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THE LOGANIACEAE OF AFRICA
I. ANTHOCLEISTA

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The present paper is the first of a series of revisions of the genera of *Loganiaceae* represented in Continental Africa. A genus is entirely revised only if the majority of the species occurs in Africa; if a small number of species is present only African representatives are taken into account.

The delimitation of the family of *Loganiaceae* is a much debated problem. The present author so far prefers to follow SOLEREDER (1892) and BRUCE & LEWIS (1960), but is of the opinion that *Retzia* which usually has been treated as an aberrant *Solanacea* belongs also in this family. After further study of the several genera constituting *Loganiaceae*, their relationship may become better understood and the family be delimited more satisfactorily. *Gaertnera*, generally regarded as a *Rubiacea*, related to *Psychotria*, is not accepted as *Loganiaceous* here.

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A REVISION OF ANTHOCLEISTA Afzel. ex R.Br.

HISTORY OF THE GENUS

Anthocleista was first described by ROBERT BROWN (1818) who adopted the name which had been written by AFZELIUS on a herbarium sheet. Its first species had been recognized by G. DON (1838). Afterwards, in the second half of the 19th century and especially shortly before and after 1900, many species were described, most of which were later reduced to synonymy, especially by Miss BRUCE (1955). The present study is based on more ample material and Miss BRUCE's conclusions regarding the delimitation of the species and their synonymy were confirmed.

GEOGRAPHICAL DISTRIBUTION

Anthocleista occurs in Continental Africa in the tropical regions, and also on Madagascar, the Comores, and the islands in the Gulf

of Guinea. Three species are endemic in Madagascar. The others are extra-Madagascan and none of them occurs throughout the area of the genus. Among those the seven "large-leaved" arborescent species are much more widely distributed than the four "small-leaved" lianas which are known from very few collections. *A. vogelii* Planch. has the largest area; it occurs from Sierra Leone to the Sudan in the North and to Angola and N. Rhodesia in the South (cf. Map 5). The highest concentration of species is between Lagos and Yaoundé, an area in which nine species occur.

The names of the countries are in accordance with the most recent map of Africa issued by the National Geographic Society. The distributional maps are from Goode's Series of Base Maps, published by the University of Chicago Press.

RELATIONSHIP WITH OTHER GENERA

Anthocleista belongs to the tribe *Potalieae* which is characterized as follows:

Potalieae (Mart.) Endl., Gen. Pl. 576. 1838.

Type genus: *Potalia* Aubl.

Homotypic synonym: *Potaliaceae* Mart., Nov. Gen. 2: 91, 133. 1827 (as *Potalieae*); Hutchinson, Fam. Flow. Pl. 2nd. ed. 1: 371. 1959 (p.p. excl. *Desfontainea*).

Heterotypic synonym: *Fagraeeae* Meisner, Gen. Pl. 1: 259. 1840 (as *Fagraeaceae*); Progel in Martius, Fl. Bras. 6(1): 267. 1868; Solereder in Engl. Prantl, Nat. Pflanzenf. 4(2): 41. 1892. Type genus: *Fagraea* Thunb.

Entirely glabrous trees, shrubs, or lianas. *Leaves* opposite, those of a pair equal or unequal, petiolate or sessile; petioles or bases of a pair usually confluent, often auriculate and/or ligulate; blade often fleshy or coriaceous, variously shaped, ovate to linear-lanceolate, entire or sometimes minutely crenate, pinnately veined. Stipules intrapetiolar and ligular, or interpetiolar and entirely connate into an ochrea. *Inflorescence* terminal or occasionally axillary, dichasial, sometimes thyrsoid, or (by reduction) racemose or spicate, 1-many-flowered. Calyx sometimes subtended by 1-several pairs of sometimes enlarged bracts; sepals 4–5, thick, fleshy-coriaceous, concave, appressed to the base of the corolla. Corolla mostly thick, fleshy, brittle, tubular; lobes 5–16, contorted (usually turned to the right) in the bud, entire. Stamens as many as the corolla lobes and alternating with them, equal; anthers ellipsoid to linear, sagittate at the base or not; cells 2, discrete, parallel, not or only at the base divergent, dehiscent throughout by a longitudinal split. Ovary superior, 2-, 4-celled, or basally 4- and apically 2-celled, with one large bilobed placenta with many ovules on both sides in each cell; style simple; stigma unbranched, capitate or saucer-shaped, sometimes bilobed. Fruit a berry, indehiscent or rarely dehiscent and 4-valved. Seeds obliquely polyhedral or ovate-orbicular, flattened or not, more or less verrucose, not winged, sometimes surrounded by a narrow ring. Embryo small,

cylindric or nearly so, surrounded by much endosperm; cotyledons short. Colleters in the axils of the leaves, bracts, and sepals.
Distribution: circumtropical; consisting of 3 genera (1 in Africa, 1 in America, and 1 in Asia and Oceania).

The tribe *Potalieae* was delimited by MARTIUS and, being a clearly demarcated natural group, is here maintained. The genera can be distinguished as follows:

1. Sepals 5; corolla lobes and stamens 5; filaments free from each other. TROPICAL ASIA, TROPICAL AUSTRALIA, and the PACIFIC ISLANDS . . . . **Fagraea**
. Sepals 4; corolla lobes and stamens 8–16; filaments entirely connate or sometimes for two-thirds of their length 2
2. Style with a globose thickening at the base; berry apically 2-celled, with few large seeds. TROPICAL SOUTH AMERICA . . . . **Potalia**
. Style not thickened at the base; berry entirely 4-celled, with many small seeds. TROPICAL AFRICA . . . . **Anthocleista**

Potalia Aubl., Hist. Pl. Guian. 1: 394, t. 151. 1775; Martius, Nov. Gen. 2: 89, t. 170. 1827; Progel in Martius, Fl. Bras. 6(1): 267. 1868; van Raalte in Pulle, Fl. Suriname 4(1): 108. 1932.
One species: *P. amara* Aubl.

Fagraea Thunb., Vet. Acad. Handl. Stockh. 3: 132, t. 4. 1782; Leenhouts, Revision of *Fagraea* in Blumea [1]).
About 60 species.

Anthocleista Afzel. ex R.Br., Exp. Tuckey Congo 30. 1818; Martius, Nov. Gen. 2: 91. 1827; Reichenbach, Consp. 133. 1828; G. Don, Gen. Syst. 4: 68. 1838; Endlicher, Gen. Pl. 577. 1838; Spach, Vég. Phan. 8: 483. 1839; Meisner, Gen. Pl. 1: 259. 1840 and 2: 168. 1840; Steudel, Nom. 2nd. ed. 1: 105. 1841; Endlicher, Ench. 289. 1841; De Candolle, Prod. 9: 36. 1845; E. Bureau, Thèse Logan. 14, 74. 1856; Bentham & J. D. Hooker, Gen. Pl. 2: 795. 1876; Solereder in Engl. Prantl, Nat. Pflanzenf. 4(2): 43. 1892; Baker in Fl. Trop. Afr. 4(1): 537. 1903; Prain & Cummins in Fl. Cap. 4(1): 1049. 1909; Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 18. 1931; Aubréville, Fl. For. Cot. Iv. 3: 153. 1936; op. cit. 2nd. ed.: 3. 184. 1959; Hasselberg, Symb. Bot. Upsal. 2(3): 40, 49. 1937; Bruce, Kew. Bull. 1955: 45; Nozeran, Nat. Monsp. Sér. Bot. 8: 167. 1957; Bruce & Lewis, in Fl. Trop. E. Afr., Loganiaceae 8. 1960.

Entirely glabrous trees, 1–35 m high, shrubs, or lianas. *Leaves* opposite, those of a pair equal or unequal, petiolate or sessile; bases or petioles joined, often auriculate, and, especially in young plants more or less conspicuously ligulate at the base; blade soft and brittle

[1]) In anticipation of publication the author kindly communicated his conclusions on the characters of this genus.

or coriaceous when living, membranaceous or papyraceous and often brittle, or coriaceous when dry, entire or minutely crenate; margin recurved or not; secondary veins conspicuous or not. Stipules intrapetiolar (ligula). *Inflorescence* terminal, erect, sometimes pendulous when in fruit, dichasial, 1–5 × branched, easily breaking at the nodes when dry. Lower bracts leafy, the others usually very small, triangular or ovate, acute or obtuse, entire. In the continental species mostly only one flower of each inflorescence open at a time (more in those of Madagascar). *Sepals* 4, green, creamy, or occasionally partially orange, free, or sometimes connate at the base (*A. laxiflora*), orbicular or nearly so, concave, decussate, appressed to the corolla tube and later to the fruit which therefore is often impressed at the base in living plants [1]), usually rounded at the apex, entire, the 2 inner ones usually becoming retuse or torn by the development of the corolla, often spreading when dry, often enlarged under the fruit. *Corolla* white, creamy, violet, violet-blue, mauve, or sometimes pale yellow, the limb often paler than the tube which is sometimes green, actinomorphic, tubular, erect in the calyx, usually not contracted when mature, thick, fleshy, brittle, also when living, often sweet-scented, soon turning brown when being shed; mature bud 1.5 – 10 × as long as the calyx; tube approximately cylindric, more or less gradually widened towards the throat, not ventricose; lobes 8–16, contorted in the bud, usually turned to the right, spreading or recurved, elliptic to lanceolate, usually obtuse, entire. *Stamens* as many as the corolla lobes and alternating with them, exserted, equal; filaments short or very short, mostly shorter than the anthers, entirely connate into a short tube or occasionally connate for two-thirds of their length, inserted near the apex of the corolla tube; anthers white or creamy, often partially green, sometimes brownish, lanceolate, obtuse or sometimes acute at the apex, usually sagittate at the base; cells 2, parallel and if sagittate only at the base divergent, dehiscent throughout by a longitudinal split. *Ovary* superior, ovoid-conic, cylindric, or obovoid-cylindric, 4-celled, usually thick-walled; style thick, about as long as the corolla tube, persisting during a short period after the corolla is shed; stigma large, usually obovoid-cylindric and apically bilobed, often slightly laterally compressed. In each cell one large bilobed placenta with numerous ovules on both sides. *Fruit* a berry, dark green or yellow, hard, globose or ellipsoid (irregular or regular indentations or furrowings are due to shrinkage and are always artificial), rounded at the apex, sometimes apiculate; wall usually thick; septa thin. *Seeds* obliquely ovoid-orbicular or irregularly polyhedral, flattened, medium to dark brown, slightly verrucose, faveolate, slightly or hardly lustrous, sometimes surrounded by a narrow ring (in some Madagascan specimens); hilum in the middle of one of the flat sides. Embryo small, nearly cylindric, slender, surrounded by much endosperm; cotyledons very short. Small colleters in one rank in the axils of the leaves, bracts, and sepals.

[1]) Living seen: *A. djalonensis* fl. fr., *A. nobilis* fl. fr., *A. procera* fr.; spirit coll.: 7 continental arborescent spp., fl. fr. and *A. obanensis* fl. buds.

Type species: *A. nobilis* G. Don.

Distribution: 14 species in tropical Africa, Madagascar, and the Comores.

All specimens studied for this revision are entirely glabrous except one, Gossweiler 8036, the leaves of which are pubescent beneath. In all other characters it agrees with *A. vogelii*. Without further collections its identity remains doubtful.

ANGOLA: between Seva and Maiombe, Gossweiler 8036 (BM, COI, K, LISU, with immature fruits).

Anthocleista may be subdivided into two groups: one with large crowded leaves and thick twigs with short internodes and one with smaller leaves and comparatively thinner twigs with long internodes.

The species with large crowded leaves are trees or few-stemmed shrubs. Those with the small leaves are as far as known usually lianas or shrubs. The latter group consists of rare species on which few field notes have been taken. Most of them were said to be terrestrial or epiphytic shrubs, some were noted as lianas, and some others were declared to be trees. If growing in open places, without support, some lianas assume the habit of shrubs (e.g. *Usteria* (Logan.) and *Abrus* (Papil., BRETELER, Blumea 1960)). Sometimes plants which were noted as trees may actually be shrubs epiphytic in trees. *A. madagascariensis*, a tree, approaches in habit the second group, because its leaves which are not distinctly crowded, but differs from all species in that group by its violet instead of creamy corolla.

The arborescent species (nrs. 1–11), and especially the continental ones, may be characterized as follows:

Trunk cylindric, sometimes with buttresses or stilt-roots, up to 70 cm in diam.; bark smooth, pale grey or pale brownish grey, not or slightly fissured when the tree is maturing; slash yellow, orange, or ochraceous, with brown stripes or not; pith thick; branches umbellate; twigs thick, with conspicuous leaf-scars, with or without spines or cushions above the leaf-axils. Trunk and branches terminating in an inflorescence. At and after anthesis the axillary buds of the upper leaves develop and give rise to some branches at the same level (umbellate branching), the tree then resembling a candelabrum (Photograph 1). Leaves crowded at the apices of the twigs (Photograph 6) (or nearly so in *A. madagascariensis* and some specimens of *A. liebrechtsiana*), usually very large, decreasing in size when the tree is maturing [1]), largest when the trunk is still unbranched, obovate, obovate-elliptic, oblong-elliptic, lanceolate, oblanceolate, or linear-lanceolate (in young plants usually comparatively narrower), variable in shape and size in a single tree, usually not more than 40×15 cm, in young plants and low-level branches up to 150×50 cm, mostly rounded or obtuse or sometimes acute at the apex, but more acute to acuminate in very young plants which have thin stems with conspicuous internodes (Figs. 6 and 10). In dense forests the trunk remains unbranched, and the tree becomes a long slender pole crowned by a cluster of large leaves and fails to flower (Photograph 2).

[1]) This character not known for *A. madagascariensis*.

The axillary buds are placed on the twigs above the leaf axils, as figured by NOZERAN (1957). If the twigs are spiny, the spines surround the buds. The developing lateral branch starts in that case with two opposite spines before producing leaves and therefore on some branches 4 spines are placed together (Fig. 7).

English names: Cabbage tree or cabbage palm; fever tree (*A. grandiflora*).

French name: Arbre à chou.

The African names do not distinguish between the species as far as known.

Madagascan name: Landemy or Landemy Lahy.

The "lianous" species (nrs. 12–14) have the following characters in common:

Unarmed shrubs, lianas, or trees (?), sometimes epiphytic. Leaves not crowded, those of a pair equal or subequal, usually shortly petiolate; blade coriaceous also when living, oblong-elliptic or oblong-obovate, up to 20 × 11 cm, usually apiculate; veins inconspicuous, margin not revolute nor recurved.

Uses: Wood rarely used as timber (*A. nobilis*, *teste* Chevalier 16196; *A. grandiflora*, *testibus* Johnston 5 et Leyson 1358), but as it is soft and the pith is thick it is not valuable. Sometimes used as fire-wood, but people sitting round the fire will become sick (*A. nobilis*, *teste* Deighton 512; *A. schweinfurthii*, *teste* Louis 7873).

Roots, bark, and leaves have medicinal value. Africans apply a decoction for various ailments, e.g.: fever (bark of *A. grandiflora*, *teste* Brady 17 Oct. 1905), chest pains (roots of *A. djalonensis*, *teste* Deighton 4215), and constipation (roots and leaves of *A. nobilis*, *teste* Deighton 512). Bark used as poultice for sores (*A. nobilis*, *teste* Cooper 190).

Key to the species:

1. Sepals acuminate and keeled. RIO MUNI. 14. **A. laxiflora**
. Sepals rounded, not keeled 2
2. Corolla violet or violet-blue, small, up to 20 mm long; lobes in mature corolla bud broadly overlapping. MADAGASCAR 3
. Corolla creamy or white, larger, usually more than 25 mm long; lobes in mature corolla bud narrowly overlapping. AFRICAN CONTINENT AND THE ISLANDS EXCEPT MADAGASCAR 5
3. Leaves papyraceous when dry, large or rather large, sessile; secondary and some tertiary veins conspicuous . . . . 3. **A. amplexicaulis**
. Leaves coriaceous when dry, smaller, petiolate or if sessile then usually more than 5 × as long as wide, and costa acute beneath; secondary veins inconspicuous. 4
4. Leaves not or not conspicuously crowded at the apices of the twigs, mostly petiolate, usually 2–3 × as long as wide and up to 17 cm long; costa not acute beneath; mature

Photograph 1. *A. procera*, tree in swamp, 6 km east of Agnéby, Ivory Coast (Leeuwenberg 3178). On the foreground *Raphia*.

corolla in the bud 15–20 mm long . . . . 1. **A. madagascariensis**
. Leaves crowded at the apices of the twigs, sessile, usually more than 5 × as long as wide and 20–80 cm long; costa triangular and acute beneath; mature corolla in the bud usually 10–12 mm long . . . . 2. **A. urbaniana**
5. Secondary veins conspicuous; leaves crowded at the apices of the twigs; candelabrum-shaped trees or few-stemmed shrubs 6
. Secondary veins inconspicuous; leaves not crowded at the apices of the twigs; lianas, shrubs or sometimes trees (?). (see also *A. liebrechtsiana*) 13
6. Branches armed with short usually paired spines; flower buds uniformly rounded or subtruncate at the apex . . . 7
. Branches unarmed; or if with incipient or occasional spines then flower buds not rounded, but tapering at the apex 9
7. Branches conspicuously spiny; spines divergent, confluent at the base, paired or sometimes 3–4 together; leaves conspicuously discolorous, dark green above, more or less glaucous beneath, usually sessile; margin usually revolute and undulate; mature corolla in the bud usually less than 5 × as long as the calyx 8
. Branches not conspicuously spiny; spines parallel or slightly divergent, confluent for at least half their length, paired; leaves not conspicuously discolorous, petiolate; margin not revolute nor undulate; mature corolla in the bud 5.5–6 × as long as the calyx. West Africa . 6. **A. djalonensis**
8. Corolla tube 0.9–1.5 × as long as the lobes and 1.25–2 × as long as the calyx; fruits drying smooth, neither shrivelled nor dented; leaves never drying black, mostly obovate and often comparatively wider than in the following species . . . . 5. **A. vogelii**
. Corolla tube 2–3 × as long as the lobes and 2.5–3.5 × as long as the calyx; fruit when dry with 4 more or less irregular dents or irregularly shrivelled; leaves often drying black. West Africa . . . . 4. **A. nobilis**
9. Leaves petiolate and buds usually uniformly rounded at the apex 10
. Leaves sessile or subsessile, if petiolate then buds not rounded, but tapering at the apex 11
10. Leaves obovate, usually rounded at the base; calyx narrowed, not definitely constricted at the mouth; branches with incipient or occasional spines; berry drying smooth, mature 35–50 × 25–35 mm, with a thick wall (about 3 mm) . . . . 6. **A. djalonensis**
. Leaves elongate-oblanceolate, always cuneate at the base; calyx definitely constricted at the mouth; branches never spiny; berry irregularly shrivelled when dry, mature 15–27 × 10–18 mm, with a thin wall (about 1 mm) . . . . 7. **A. liebrechtsiana**

Photograph 2. In the centre *A. nobilis*, 25 km north of Taï, Ivory Coast, along road to Guiglo.

11. Sepals drying smooth, clasping the corolla tube at anthesis, only spreading under the mature fruit; leaves, at least the upper ones, usually petiolate, lower ones often sessile or subsessile, usually subcoriaceous, and with inconspicuous tertiary veins; corolla tube about 1–1.5 × as long as the lobes which are large and reflexed 8. **A. schweinfurthii**
. Sepals drying rugulose, outer pair at least more or less spreading, not closely clasping the corolla tube at anthesis, widely spreading under the fruit; leaves sessile or subsessile, usually membranaceous, and with conspicuous tertiary veins; corolla tube 1.5–2.5 × as long as the lobes, which are smaller and spreading or sometimes recurved 12
12. Dry berry umbonate at the apex, at least when young; corolla tube 1.5–2.5 × as long as the calyx. East and South Africa and Comores . . . . 9. **A. grandiflora**
. Dry berry rounded or truncate at the apex; corolla tube 3.5–5.5 × as long as the calyx. West Africa . . . . 10. **A. procera**
13. Outer sepals about as long as wide or wider 14
. Outer sepals 1.5–2 × as long as wide, 12–17 × 8–11 mm, often apically torn . . . . 13. **A. obanensis**
14. Sepals 20–30 mm long . . . . 12. **A. scandens**
. Sepals 4–8 mm long . . . . 11. **A. microphylla**

1. **A. madagascariensis** Bak., Journ. Bot. 20: 173. 1882.
Fig. 1; Map 1

Type: Madagascar: Betsileo District, Ankafina, Baron 73 (K, holotype; isotype: P).

Heterotypic synonyms: *A. rhizophoroides* Bak., Journ. Linn. Soc. 22: 506. 1887. Lectotype: Madagascar: sin. loc., Baron 3814 (K, lectotype; isotypes: P, 3 sheets).

A. hildebrandtii Gilg in Engl. Bot. Jahrb. 17: 584. 1893. Type: Madagascar: Betsileo District, Nandihizana, Hildebrandt 3899a (holotype destroyed in B; isotype: K!).

Tree 3–15 (usually about 8–12)m high, without spines. Leaves petiolate or sometimes sessile; blade dark green above, paler beneath, coriaceous and dull or hardly shiny on both sides when dry, oblong-obovate or oblanceolate, 1½–3¼ (usually about 2–3) × as long as wide, 5–17 × 2–9 cm (up to 24 × 11 cm or more (?)), decurrent into the petiole; costa prominent but not acute beneath; secondary veins inconspicuous; margin usually revolute when dry and not well pressed. Sepals creamy or pale green, rounded, when dry more or less rugulose outside and often more or less spreading, the outer ones orbicular or broadly ovate, 5–9 × 6–9 mm, the inner ones not or slightly larger and becoming retuse by the development of the corolla. Corolla in the young bud rounded or slightly tapering and apiculate at the apex, in the mature bud 2–4 × as long as the calyx, 15–20 mm

long, and more or less rounded, violet; tube 1–2 × as long as the calyx, 1–1½ × as long as the lobes; lobes 10, orbicular or elliptic, cordate or rounded at the base, spreading. Berry when dry ellipsoid, smooth, shining, apiculate, thick-walled.

Trunk up to 30 cm in diam. or more(?); twigs when dry about 5–8 mm in diam. *Leaves* not conspicuously crowded, those of a pair equal or unequal, the larger one up to about twice as long as the smaller; petiole up to ¼ × as long as the blade, usually 1–2.5 cm, up to 4 cm long, widened, slightly auriculate, and inconspicuously ligulate at the base; blade rounded at the apex, entire; secondary veins 5–9 pairs. *Inflorescence* 3–5 × branched, 5–20 (usually about 10–15) cm long. Peduncle, branches, and pedicels creamy or pale green, not thickened. Upper bracts triangular, 1–2 × as long as the diameter of the corresponding branch, acute. Outer sepals 0.62–1.25 × as long as wide, the inner ones about as long as wide or wider. Corolla tube cylindric or nearly so, short, 8–12 mm long, not contracted but often seemingly so by drying, 3–5 mm wide at the base and at the throat; lobes 1–1½ × as long as wide, 7–10 × 7–8 mm, obtuse or rounded at the apex. Filaments entirely connate; anthers brownish-white, about 4 × as long as wide, 4-6 × 1–1.5 mm, with an often large petal-like sterile acute apex, shortly sagittate at the base. Pistil green. Ovary obovoid, about 2 × as long as wide, 5–6 × 2.5–3 mm, thick-walled; stigma obovoid, about 1½ × as long as wide and as wide as thick, 2–2.5 × 1.5–2 × 1–1.5 mm, apically retuse. Dry berry 1.2–2 × as long as wide, 2.5–4 × 1.5–2.5 cm, hard. Seeds orbicular, obliquely ovate, or ellipsoid, 1–1½ × as long as wide, 2–2.5 × 1.5–2.5 mm, medium brown, usually surrounded by a narrow ring. Embryo about 1 mm long.

Distribution: Madagascar.

Ecology: In open moist (?) places, mostly in rain forests. Alt. 450–1700 m or less (?).

MADAGASCAR: Ambatolana, Herb. Tananarive 4375d (P); Marojejy Mts., Cours 3584 (P); ibid., Humbert 22587 (P), 23018 (P); near Bealanana, Ankaizina, Perrier de la Bâthie 15081 (P); Sofia R., near Antsakabary, Humbert 18094 (G, P); Masoala, East Coast, Perrier de la Bâthie 10261 (P); Maroambitsy, Service des Eaux et Forêts 9765 (P); Amberimay, between the Mahajamba and Bemarivo Rs., Perrier de la Bâthie 10214 (P); Alaotra Lake, between Menasaka and Ambodriana, banks of Maningory R., Homolle 487 (P); Ambohimirahavavy Mts., Service des Eaux et Forêts (Capuron) 967 (P); between Antoby and Ambositra, Service des Eaux et Forêts 6357 (P); Mandraka, east of Tananarive, Perrier de la Bâthie 14593bis (P); between Manohaka and Manjakandriana, Service des Eaux et Forêts 10292 (P); between Vohitraramy and Moramanga, Service des Eaux et Forêts 7595 (P); south of Moramanga, Decary 7017 (P); Forêt d'Analamazaotra, near Perinet, Moramanga District, Andovoranto Province, Viguier & Humbert 1115 (B, P); ibid., Thouvenot 68 (P); ibid., Perrier de la Bâthie 8622 (P); Perinet, east of Moramanga, Service des Eaux et Forêts 15011 (P); ibid., Reserves Naturelles (Saboureau) 1254 (P); ibid., Service des Eaux et Forêts 4238 (P), 7609 (P); Antanjona, Alleizette 726M (P); Jarahina, near boundary of Sihanaka District, Herb. Tananarive 2732 (P); between Vatondrangy and Tandriana, Service des Eaux et Forêts 14830 (P); km 56, road Anosibé–Namorana–Moramanga, Service des Eaux et Forêts 7837 (P); Ambatolampy, Service des Eaux et Forêts 1010(P); between Tsinjoarivo and Ambatolampy, Service des Eaux et Forêts 3934 (P); road Ambomitzara–Anjamana–Ambatolampy, Service des Eaux et Forêts 13008 (P); near Ambositra, Forêt de Ranomena, Humbert & Swingle 4861 (P); Betsileo District, Ankafina, Baron 73 (K, P, type); Betsileo District, Nandihizana, Hildebrandt 3899a (K, isotype of *A. hildebrandtii*); near Fort-Carnot, Service des Eaux et Forêts 5451 (P); Ivakoany Mts., Humbert 6989 (P), 12196 (G, P); Upper Mandrare R., Marosousi Mt., Humbert 6628 (P); Ampanatonampingotra, Andapa, Service des Eaux et Forêts 3662 (P); Forêt d'Analamihilana, Cours 2031(P); Ambalavoanio, Mainampango Mt., Cours 3216 (P); Anjahanaribe, Cours 3721 (P); sin. loc.: Baron 1283, p.p. (P), 1357 (K), 1952 (K, paratype of *A. rhizophoroides*), 3814 (K, P, 3 sheets, lectotype of *A. rhizophoroides*), 5097 (K).

A comparison of the types of *A. madagascariensis*, *A. rhizophoroides*, and *A. hildebrandtii*, and the other specimens cited, shows that all belong to a single species. The range of variation within this species is much smaller than in most of the continental species.

Map 1. *A. madagascariensis;* Map 2. *A. urbaniana;* Map 3. *A. amplexicaulis.*

2. **A. urbaniana** Gilg in Engl. Bot. Jahrb. 17: 584. 1893.

Fig. 2; Map 2

Type: Madagascar: sin. loc., Humblot 662 (holotype destroyed in B; isotypes: K!, P, 5 sheets!).

Tree, 3–7 m high or more (?), without spines. Leaves sessile or subsessile; blade medium (?) to dark (?) green above, paler and with pale costa beneath, when dry brownish to brownish-green, dull or slightly shiny on both sides, coriaceous, rather brittle, oblanceolate to linear, very variable in shape and size, 3–15 × as long as wide, 20–80 × 2–21 cm, narrowed to the base; costa sharply triangular beneath; secondary veins more or less inconspicuous. Sepals white, rounded, when dry dull, minutely rugulose, and usually clasping the corolla or the fruit, the outer ones orbicular or broadly ovate, 5–9 × 4–8 mm, the inner ones usually slightly larger, becoming retuse by the development of the corolla. Corolla in the young bud rounded at the apex, usually included in the calyx, in the mature bud 1.5–1.8 × as long as the calyx, 10–14 mm long, and rounded, violet; tube 0.6–1 × as long as the calyx, about as long as the lobes; lobes 9–11, ovate-elliptic, rounded at the base, spreading. Dry berry rather hard, globose or nearly so, rounded or acuminate at the apex, more or less rugulose, rather thick-walled.

Twigs about 5–15 mm in diam. when dry. *Leaves* of a pair subequal or unequal; base widened and obscurely ligulate; blade obtuse to acuminate at the apex; costa prominent beneath; secondary veins 10–16 pairs. *Inflorescence* 4–6 × branched, 10–24 cm long, usually broader than long. Peduncle, branches, and pedicels white, not thickened. Upper bracts oblong-triangular to triangular-lanceolate, 2–6 × as long as the diameter of the corresponding branch, $1\frac{1}{2}$–4× as long as wide, up to 25 mm long, obtuse. Outer sepals 1–1.2 × as long as wide. Corolla tube approximately infundibuliform, 4–8 mm long, not or slightly contracted, widened towards the throat, 2.5–3 mm wide at the base, 2.5–5 mm at the throat; lobes about 1.2–1.5 × as long as wide, 6 × 4–5 mm, obtuse at the apex. Filaments entirely connate; anthers 3–4 × as long as wide, 3.5–4 × 1–1.5 mm, apically sterile or not, sagittate at the base. Ovary comparatively large, ovoid-conic, about

2 × as long as wide, 4–5 × 2–3 mm, thick-walled; stigma ovoid-conic, about 1½ × as long as wide, not retuse. Dry berry 0.75–1.2 × as long as wide, 15–20 × 15–20 mm. Seeds medium or dark brown, obliquely polyhedral or approximately orbicular-ovate, sometimes surrounded by a narrow ring, 1–1½ × as long as wide, 2.5–3.5 × 2–2.5 mm.

Distribution: Madagascar.

Ecology: In rain forests or secondary forests, in open (?) places. Alt. 0–500 m.

Madagascar: Maroantsetra, Anonymus 149 (P); Soanierana-Ivongo, Service des Eaux et Forêts 2365 (P); 2369 (P); between Soanierana-Ivongo and Antasibe, Lam & Meeuse 5805 (L); Mananjary Province, Geay 7189 (P), 7190 (P), 7695 (P); ibid., Vatovavy Mt., Decary 13686 (P); Lower Matitanana R., Perrier de la Bâthie 2036 (P); Karianga, Farafangana Province, Decary 5656 (P); Mandena, north of Fort-Dauphin, Service des Eaux et Forêts (Capuron) 393 (P); Col d'Ivolo, Fort-Dauphin District, Decary 10549 (P); Nahampoana, Fort-Dauphin District, Decary 10786 (P); Manantantely, near Fort-Dauphin, Humbert 5731 (P); Ankatsaka, Antalaha, Service des Eaux et Forêts 5038 (P); sin. loc.: Commerson s.n. (P); Humblot 662 (K, P, 5 sheets, type); de Lastelle anno 1841 (P); Chapelier anno 1813 (BM).

3. **A. amplexicaulis** Bak., Journ. Linn. Soc. 22: 506. 1887.
Fig. 3; Map 3

Type: Madagascar: sin. loc., Baron 3795 (K, holotype; isotypes: K, P, fragm.).

Tree 3–15 m high, without spines. Leaves sessile; blade dark green above, paler beneath, brittle, when dry greenish-brown, dull or shining, papyraceous to subcoriaceous, obovate to oblanceolate or sometimes linear-lanceolate, 1½–4(6) × as long as wide, 7.5–90 × 3.5–35 cm; costa often acute beneath; veins conspicuous; margin not revolute nor recurved. Sepals whitish-green or green, rounded, when dry conspicuously rugulose or not and more or less spreading, the outer ones orbicular or broadly ovate, 4–10 × 4–10 mm, the inner ones becoming emarginate by the development of the corolla. Corolla in the young bud at the apex as in the mature bud or rounded, in the mature bud about 1.6–2.5 × as long as the calyx, 10–19 mm long, more or less tapering and often apiculate at the apex, violet or mauve; tube 0.8–1.25 × as long as the calyx, 0.8–1 × as long as the lobes; lobes 8–12, orbicular, broadly ovate, or elliptic, cordate or rounded at the base, spreading. Dry berry ovoid, subglobose, ellipsoid, or fusiform, smooth and shining to conspicuously rugulose, apiculate or acuminate, with a thick or rather thick wall.

Trunk 15–20 cm in diam.; twigs about 1–3 cm in diam. when dry. *Leaves* of a pair subequal; base often auriculate; blade rounded at the apex, narrowed to the base, entire; costa and veins prominent beneath; secondary veins 8–17 pairs. *Inflorescence* 4–6 × branched, 15–30 cm long or more (?). Peduncle, branches, and pedicels greenish-white, not or slightly thickened at the nodes. Upper bracts triangular or oblong-triangular, 1–6 × as long as the diameter of the corresponding branch, 1–3 × as long as wide, obtuse, acute, or acuminate. Outer sepals 0.8–1.2 × as long as wide, the inner ones usually slightly larger. Corolla tube cylindric to amply infundibuliform, 5–9 mm long, not contracted, 2–5 mm wide at the base, 3–8 mm at the throat; lobes 1–1.5 × as long as wide, 5–10 × 4–7 mm, obtuse or rounded at the apex. Filaments usually entirely connate, sometimes for about

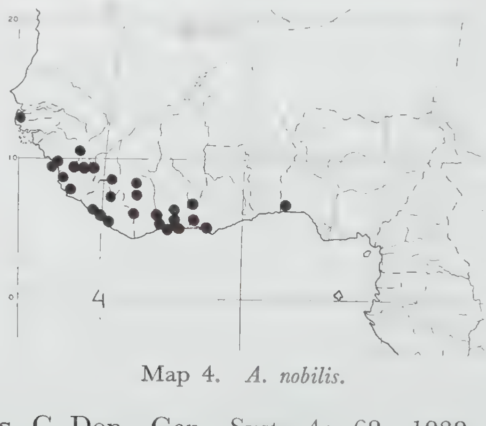
two-thirds of their length; anthers sometimes reported as brownish, about 3–4 × as long as wide, 3.5–6 × 1–1.5 mm, with a sterile acute apex, truncate or shortly sagittate at the base. Ovary ovoid or oblong-ovoid, about 2 × as long as wide, 5–6 × 2.5–3 mm, thick-walled; style short or very short; stigma globose or obovoid, about 1.5–2 × 1.5 mm, retuse at the apex. Berry green, rather hard, when dry 1.3–2.5 × as long as wide, 20–30 × 11–22 mm. Seeds medium brown, obliquely ovate-orbicular or polyhedral, 1–2 × as long as wide, 2.3 × 1.5–3 mm. Embryo 1.5 × 0.15 mm.

Distribution: Madagascar.

Ecology: Usually (?) in swamps, in forests. Alt. 0–1200 m.

MADAGASCAR: Ambre Mt., Service des Eaux et Forêts 686 (P); Soanierana-Ivongo, Ambohoabé, Lam & Meeuse 5666 (L, WAG); Forêt d'Analamaitso, Upper Bemarivo R., Perrier de la Bâthie 10200 (P); north of Ankazobé, Decary 7393 (P); Manerinerina, between Tampoketsa and Ankazobé, Decary 17241 (P); between Ampitanonoka and Fotsianana, Cours 2463 (P); between Ambonidobo and Moramanga, Service des Eaux et Forêts 7388 (P); Angavotzety (?), Carion Canton, Manjakandriana District, Reserves Naturelles (Saboureau) 1541 (P); Mandraka, Alleizette 685 (P); Forêt d'Analamanity, Imerina, Camboué 1 (P), 2 (P), 12 (P); Forêt d'Analamazaotra, near Perinet, Moramanga District, Andovoranto Province, Viguier & Humbert 1119 (B, P); ibid., Perrier de la Bâthie 8600 (P), 8601 (P), 8620 (P), 15977 (P); Anosibé, south of Moramanga, Decary 18304 (P); between Ampotaka and Moramanga, Service des Eaux et Forêts 6611 (P); Ranomena, near boundary of Sihanaka District, Herb. Tananarive 2721 (P); Midongy du Sud, near Farafangana, Decary 4919 (P); sin. loc.: Baron 1283, p.p. (K, P), 1284 (BM, K, P), 3795 (K, 2 sheets, P, type); Humblot 496 (K, P).

Although this species is far more variable than both other Madagascan species, especially in the shape and size of the inflorescence, peduncle, branches, pedicels, and fruit, it is easily recognized by its large, usually rather thin leaves with conspicuous veins.



Map 4. *A. nobilis.*

4. **A. nobilis** G. Don, Gen. Syst. 4: 68. 1838; De Candolle, Prod. 9: 36. 1845; Baker, Fl. Trop. Afr. 4(1): 538. 1903, p.p. excl. syn. *A. vogelii* Planch., *A. buchneri* Gilg, *A. niamniamensis* Gilg et *A. macrantha* Gilg; Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 18. 1931, p.p. excl. syn. *A. djalonensis* A. Chev.; Aubréville, Fl. For. Cot. Iv. 3: 154, pl. 301. 1936; op. cit. 2nd. ed. 3: 186, pl. 318. 1959; Bruce, Kew Bull. 1955: 47.

Figs. 4-6; Map 4

Lectotype: Sierra Leone: sin. loc., Don s.n. (BM).

Heterotypic synonyms: *A. macrophylla* G. Don, l.c.; De Candolle, l.c. Cult. in garden, no type specimen preserved.

A. parviflora Bak., Kew Bull. 1895: 99; et l.c. p. 539. Type: Sierra Leone: Bagroo R., Mann anno 1861 (K, holotype; isotype: P).

Tree 4–30 (usually up to 18) m high. Twigs with 2 spines above the leaf axils which are divergent and confluent at the base. Leaves sessile or shortly petiolate; blade dark green and slightly shining above, pale glaucous and dull beneath, usually drying dark brown to black, especially above, coriaceous when living, brittle, drying coriaceous to papyraceous, oblong-elliptic, obovate-elliptic, or oblanceolate, 1½–4 (usually about 3, in young plants up to 4½(–8½) × as long as wide, 6.5–35 × 4–12 cm, up to 150 × 35 cm in young plants, usually long-decurrent into the petiole or narrowed to the base; margin often largely undulate and usually recurved. Sepals creamy or pale green, rounded, when dry more or less rugulose and appressed to the corolla, the outer ones orbicular or ovate-elliptic, 7–10 × 4.5–13 mm, the inner ones slightly larger or smaller, often becoming retuse by the development of the corolla. Corolla in the mature bud 3.75–5 × as long as the calyx, 30–39(45) mm long, and as in the young bud rounded or subtruncate at the apex, white or creamy; tube 2½–3½ × as long as the calyx, 2–3(4) × as long as the lobes; lobes 11–14, oblong, spreading. Berry ellipsoid, rounded, when dry irregularly shrivelled and with 4 more or less irregular dents, rounded; wall thick.

Trunk without buttresses, 15–90 (usually up to 45) cm in diam.; twigs about 1–3 cm in diam. *Leaves* of a pair subequal or sometimes unequal; petiole up to $^1/_6$ × as long as the blade, auriculate at the base, blade in young leaves soft, pale green and shining on both sides, mostly rounded at the apex, acuminate to obtuse in young plants; costa and veins prominent beneath; secondary veins 5–18 pairs. *Inflorescence* 3–6 × branched, 12–60 cm long and wide. Peduncle, branches, and pedicels creamy or green, thickened at the nodes. Upper bracts triangular, acute, about as long as the diameter of the corresponding branch or longer. Outer sepals 0.75–1.25 × as long as wide, inner sepals 8–10 × 7–11 mm. Corolla tube nearly cylindric, 21–31 mm long, contracted at the base when young, gradually widened towards the throat, 5–7 mm wide at the base, 10–12 mm at the throat; lobes 2.25–2.5 × as long as wide, 9–12 × 4–5 mm, obtuse or rounded. Filaments entirely connate; anthers about 4–5 × as long as wide, 5–6 × 1–1.5 mm, shortly sagittate at the base. Ovary obovoid, about 2 × as long as wide, 6–7 × 3–4 mm, thick-walled; stigma obovoid-cylindric, about 1–1.5 × as long as wide, 3–3.5 × 2.5–3 mm, apically emarginate. Berry dark green, hard, about 1.2–1.5 × as long as wide, 3–4 × 2–2.5 cm. Seeds dark brown, obliquely ovate-orbicular, 1.25–1.75 × as long as wide, 2–2.5 × 1.5–1.7 mm. Embryo about 1 × 0.15 mm.

Distribution: West Africa, from Sénégal to S.W. Nigeria.

Ecology: In secondary forests and on road sides in rain forests or on coastal savannas in the rain forest region. Usually at low elevations. Alt. 0–1200 m.

SÉNÉGAL: between Bignona and Sindialone, Casamance, Chevalier 21–23 Feb. 1900 (P).

GUINEA: Los Islands, Chevalier 12250 (P, cited by Chevalier as *A. procera* var. *umbellata*); road of Conakry, Maclaud 28 (P); Ymbo, bank of Kosikouré R., Chevalier (Caille) 14759 (P, photograph: K, cited by Chevalier as *A. procera* var.

parviflora); between Santa R. and Timbo, Chevalier 12613 (P, photograph in K, cited by Chevalier as *A. procera* var. *umbellata*); Macenta, Roberty 7150 (G).

Sierra Leone: Mt. Loma, Jaeger 1577 (K), 1641 (K); Talla Hills, Scott Elliot 5053 (K, BM); Karene District, Scott Elliot 5872 (K); Freetown, Dalziel 984 (BOL, K, Z); Sugarloaf Mt., Barter 5–57 (K); Njala, Deighton 512 (K, BM); Bagroo R., Mann anno 1861 (K, P, type of *A. parviflora*); Kangahun, Deighton 6146 (K); sin. loc.: Afzelius anno 1798 (Linn–J. E. Smith 1148, "*A. nigrescens*"); s.n. (BM); G. Don s.n. (BM, type); Hepper 2526 (K); C.E.L.P. 15, Sierra Leone Herb. (K); Thomas anno 1914 (K).

Liberia: Gbanga, Linder 542 (K); Peahtah, Linder 1021 (A, K); Gbarnga, Okeke 243 (MO, BM, BR, COI, G); Monrovia, Dinklage 2420 (B); Dukwai R., Monrovia, Cooper 190 (BM, GH, K, S), 380 (BM, GH, K), 428 (GH, K); Grand Bassa, Baldwin 11197 (K); sin. loc., Harley 637 (K).

Ivory Coast: Mt. Tonkouï, diapositive in Wageningen, taken by Leeuwenberg; 75 km E. of Toulépleu, Photograph 3; 25 km N. of Taï, Photograph 2; 52 km E.N.E. of Sassandra, Leeuwenberg 3084 (WAG, juv.); Agboville, Chevalier 22340 (P, WAG); Yapo, Roberty 12118 (G, juv.); Dabou, Chevalier 16196 (P); ibid., Leeuwenberg 2322 (WAG, with fl. in spirit coll., BR); between Dabou and Akouaho, Jolly 41 (P); Adiopodoumé, 17 km W. of Abidjan, Herb. I.D.E.R.T. 3172 (ABI); ibid., Leeuwenberg 1921 (WAG, BR, K, LISC, MO, P, Z); Banco, Aubréville 407 (P); Abidjan, Chevalier 17327 (P); Bingerville, Chevalier 16094 (P); ibid., Leeuwenberg 3168 (WAG, UC); ibid., de Wilde 496 (WAG); Aboisso, Chevalier 16291 (P); near Abé, station of Makougnié, Chevalier 16156bis (P, K); sin. loc., Aubréville 922 (P).

Ghana: Jemma, W. frontier, Chipp 354 (K); 25 km N.W. of Prestea, Vigne 1270 (K); near Axim, Irvinge 2198 (E, K); ibid., Morton A 2221 (K).

Nigeria: Ibadan, Onochie FHI 34913 (K).

Map 5. *A. vogelii.*

5. **A. vogelii** Planch. in W. J. Hooker, Icon. 8: tt. 793–794. 1848; W. J. Hooker, Niger Fl. 459, t. 43–44. 1849; Solereder in Engl. Prantl, Nat. Pflanzenf. 4(2): f. 24 A–E. 1892; Hutchinson & Dalziel,

Photograph 3. *A. nobilis*, young trees, 75 km east of Toulépleu, along road to Guiglo, Ivory Coast.

Photograph 4. *A. djalonensis*, flowering and fruiting branch, 25 km west of Bouaké, Ivory Coast (Leeuwenberg 3285 of which seedlings in the greenhouse in Wageningen).

Photograph 5. Young plants of *A. djalonensis* (Leeuwenberg 3167) on foreground and *A. nobilis* on background right side, east of Bingerville, Ivory Coast; edge of coffee plantation.

Photograph 6. *A. procera*, fruiting branch (Leeuwenberg 3178).

Fl. W. Trop. Afr. 2: 18. 1931; Aubréville, Fl. For. Cot. Iv. 3: 154. 1936; op. cit. 2nd. ed. 3: 184. 1959; Bruce, Kew Bull. 1955: 48; Bruce & Lewis, in Fl. Trop. E. Afr., Loganiaceae 8. 1960.

Fig. 7; Map 5

Type: Nigeria: Niger Delta, Aboh (Ibu), T. Vogel 51 (K, 3 sheets).

Heterotypic synonyms: ? *A. buchneri* Gilg in Engl. Bot. Jahrb. 17: 576. 1893. Type: Angola: Lunda, banks of the Luachimo R., Buchner 618 (holotype not seen, destroyed in B). Topotype: Marques 267 (COI, LISU = *A. vogelii*!).

A. kalbreyeri Bak., Kew Bull. 1895: 99; in Fl. Trop. Afr. 4(1): 540. 1903. Type: S. Nigeria: Banks of R. Bonny, Kalbreyer 61 (BM, holotype).

A. zenkeri Gilg in Engl. Bot. Jahrb. 28: 124. 1899; Baker, l.c. p. 539; De Wildeman, Bull. Herb. Boiss. Sér. 2.1: 831. 1901. Type: Cameroun: Bipindi, Zenker 1965 (holotype not seen, destroyed in B; isotypes: A!, BM!, BR, 2 sheets!, COI!, E!, G!, GOET!, HBG!, K!, L!, M!, MO!, P, 2 sheets!, S!, WU!, Z!).

? *A. lanceolata* Gilg in op. cit. 28: 125. 1899; Bruce, Kew Bull. 1955: 57. Type: Cameroun: Batanga, Dinklage 1114 (holotype not seen, destroyed in B; no isotype seen).

A. auriculata De Wild., Ann. Mus. Congo Sér. 5. 3: 250, t. 31. 1910. Type: Congo: Equateur, near Coquilhatville, Eala, Peynaert 937 (BR, 3 sheets; photographs of 2 sheets in K).

A. talbotii Wernham in Cat. Talbot's Nigerian Pl. 68. 1913. Lectotype: S. Nigeria: Calabar, Oban, Talbot 2037 (BM, lectotype; isotype: K).

A. bequaertii De Wild., Rev. Zool. Afr. 10. Suppl. Bot. 8. 1922. Type: Congo: Orientale, Irumu, Bequaert 4904 (BR, 2 sheets; photograph of one sheet in K).

A. macrantha Gilg in Engl. Bot. Jahrb. 17: 578. 1893; Hiern, Cat. Welw. Afr. Pl. 1: 700. 1898. Lectotype: Angola: Golũngo Alto, near Quilombo, Bumba and Queta, Welwitsch 6021 (BM, lectotype; isotypes: BM, 4 sheets, C, COI, G, K, LISU, 8 sheets, P). Aberrant large-flowered form (see remarks).

Misapplied name: *A. nobilis* Baker, l.c. p. 538, quoad spec. Vogel 51, non G. Don.

Tree, 6–20 m high or more (?). Twigs with 2(–4) spines which are divergent and confluent at the base or occasionally unarmed. Leaves sessile or very shortly petiolate; blade dark green and often glossy above, pale glaucous beneath, when dry dark brown above, paler beneath, brittle, papyraceous to coriaceous, oblong-obovate or oblanceolate, in young plants usually narrower, 1.75–3.5 (usually about 2, in young plants up to 4) × as long as wide, usually broader than in *A. nobilis*, 15–45 × 6–24 cm, up to 150 × 45 cm in young plants, narrowed to the auricles or decurrent into the petiole, if petiolate cordate at the very base; margin usually recurved as in *A. nobilis*. Sepals pale green, occasionally partially orange, rounded, when dry more or less rugulose and somewhat spreading or not, the outer ones orbicular or broader than long, 1–1½ × as broad as long,

4–12 × 7–15 mm, the inner ones usually larger, up to about 2 × as long as the others, often partially torn by the development of the corolla. Corolla in the young bud at the apex as in the mature bud or sometimes obtuse, in the mature bud 2½–4 × as long as the calyx, 23–37 mm [1]) long, and rounded or subtruncate at the apex, creamy or sometimes pale yellow, the tube darker than the lobes; tube 1.25–2 × as long as the calyx, 0.9–1.5(1.7) × as long as the lobes; lobes 13–16, oblong-lanceolate, spreading. Berry thick-walled, globose or ellipsoid, rounded at the apex, when dry and mature occasionally apiculate, but not shrivelled, occasionally so when dry and immature.

Trunk 15–55 cm in diam., sometimes with stilt-roots; twigs about 1–2 cm in diam. *Leaves* of a pair subequal or sometimes unequal; petiole—if present—about $^1/_{30}$ × as long as the blade, auriculate at the base; blade shining on both sides when young, mostly rounded at the apex, acuminate to obtuse in young plants; costa and veins prominent beneath; secondary veins 9–22 pairs. *Inflorescence* 3–5 × branched, about 30–50 cm long and wide. Peduncle, branches, and pedicels sometimes yellowish-green or orange, thickened at the nodes. Upper bracts triangular, 1–2 × as long as the diameter of the corresponding branch, acute or rounded. Corolla tube amply infundibuliform, 12–18 mm long, contracted at the base when young, gradually widened towards the throat, 5–10 mm wide at the base, 13–16 mm at the throat; lobes 2–3 × as long as wide, 12–19 × 5–10 mm, obtuse. Filaments entirely connate or sometimes only for two-thirds of their length; anthers whitish-green, 4–5 × as long as wide, 6–8 × 1.5–2 mm, shortly sagittate at the base. Ovary ovoid-cylindric or ovoid-conical, about 1–2 × as long as wide, 5–7 × 2.5–6 mm, thick-walled; stigma obovoid-cylindric, about 1½ × as long as wide and slightly laterally compressed, 3–6 × 2–4.5 × 2–3 mm, apically bilobed. Berry green or yellowish (*teste* Milne-Redhead 2846), about 1–1.3 × as long as wide, 2.5–4.5 × 2–3.5 cm. Seeds dark brown, obliquely ovate-orbicular, about 1–1.7 × as long as wide, about 2–2.5 × 1.5–2 mm. Embryo 0.6 × 0.2 mm.

Distribution: Tropical Africa, from Sierra Leone to Uganda in the North and Angola and Northern Rhodesia in the South.

Ecology: Usually in moist places, in swamps, in Raphia groves, on river banks; in primary rain or secondary forests. Alt. 0–1500 m.

SIERRA LEONE: near Kolia, Deighton 3204 (BR, K, juv.); Kangahun, Deighton 6148 (K, with fr. in spirit coll.); ibid., Jordan 2131 (K, with fl. buds in spirit coll.); Baoma, Deighton 3203 (K, juv.).

LIBERIA: Mecca, Boporo District, Baldwin 10435 (K); Gbanga, Linder 533 (A, K).

GHANA: Kwaben, Vigne 1295 (OXF ?, not seen, cited by miss Bruce).

NIGERIA: Ibadan, Onochie FHI 40448 (BR, K, with fl. and fr. in spirit coll.); Oyo Road near Mile 116, side of Ibadan, Meikle 1311 (K, with fl. in spirit coll.); Lagos, Bels 28 (U); Lagos Lagoon, Ejinrin, Onochie FHI 34142 (K); Benin City, Palisot de Beauvois XVII (G); Warri (?) and Benin Provinces, Palisot de Beauvois s.n. (G); Niger Delta, Barter 2104 (K, P); Aboh (Ibu), Niger Delta, Vogel 51 (K, 3 sheets, type); banks of Nun R., Mann 471 (GH, K, P); banks of Bonny R., Kalbreyer 61 (BM, holotype of *A. kalbreyeri*); Akamkpa Rubber Estate, Dukwe Division, Calabar R., Calabar District, Latilo FHI 41311 (K); Oban, Calabar Province, Talbot 177 (BM, paratype of *A. talbotii*), 2077 (BM, K, lectotype of *A. talbotii*).

CAMEROONS: Ambas Bay, Mann XVI (K, P); Victoria District, Maitland 362 (K); near Victoria, Deistel 181 (M).

OUBANGUI-CHARI: between Dékoua and Nana R., Chevalier 6221 (P).

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[1]) In Welwitsch 6021: corolla: mature bud up to 47 mm long; tube 26 mm long; throat 22 mm wide; lobes 21 mm long.

Cameroun: Kongola, Mbassa, Mildbraed 9107 (K); Compenda, near Mundame, Büsgen 23 (B); between Foumban and Banyo, Jacques Félix 3163 (P); Yaoundé, Foury 97 (P); Bipindi, Zenker 1965 (A, BM, BR, 2 sheets, COI, E, G, GOET, HBG, K, L, M, MO, P, 2 sheets, S, WU, Z, isotypes of *A. zenkeri*).

Gabon: near Libreville, Chevalier 33630 (P); ibid., Jolly 145 (P); ibid., Klaine 28 (P); near Kango, Komo R., Chevalier (Fleury) 26683 (P, WAG); Lake Anengue, SE of Port Gentil, Krukoff 120 (A); Achouka, Dybowski 113 (P); Tchibanga, Le Testu 1084 (IFAN, P); sin. loc., Duparquet s.n. (P).

Moyen Congo: Kouilou R., Sargos 120 (P).

Congo: Leopoldville: Nioki, Kutu Territory, Flamigni 9039ter (BR); Luki R., Moanda, Boma, Wagemans 660 (BR); between Boma and Bananene, Wagemans 176 (BR, K); Mayombé, de Briey 230 (BR); ibid., Panga Munga, Gossweiler 6482 (BM, COI); Bundi R., Matadi Territory, Compère 356 (BR); near Pic Cambier, near Matadi, Dacremont 311 (BR); Samu Zambi, mouth of Congo R., Donis 2401 (BR); Wamba R., Dibaya Territory, Liben 3184 (BR); Kwango R., Devred 2513 (BR, WAG); ibid., Kahemba, Devred 2993 (BR).
Equateur: Bodangabo, Gbelu R., east of Gemena, Evrard 264 (BR); Dundusana, Bumba Territory, Mortehan 690 (BR); Ngiri R., Bomboma Territory, Léontovitch 73 (BR, WAG); Eala, near Coquilhatville, Corbisier-Baland 1235 (B, BR, K, P), 1960 (BR); ibid., Pynaert 937 (BR, 3 sheets, type of *A. auriculata*); between Bikoro and Coquilhatville, Germain 8420 (BR, K); Befale Territory, Evrard 3481 (BR, WAG), 5124 (BR); Monkoto, Evrard 4452 (BR, WAG); Ingende Territory, Evrard 6087 (BR, WAG).
Orientale: Monbuttu, Munsa, near Niangara, Schweinfurth 3376 (K); between Yambao and Mongandjo, Limbete R., Evrard 2108 (BR); Yangole, west of Yangambi, Louis 12023 (BR, juv.); Gazi, Louis 10953 (BR); ibid., Ikele R., Louis 13544 (BR, WAG); Yangambi, Germain 79 (BR); ibid., Louis 16411 (BR); ibid., A. Léonard 1146 (BR); left bank Yandja Lake, Isangi Territory, Germain 422 (BR, WAG); Irumu, Bequaert 4904 (BR, 2 sheets, type *A. bequaertii*).
Kivu: Loso R., Masisi Territory, Ghesquière 4943 (BR, WAG); Bukombo, Masisi Territory, Gutzwiller 829 (BR), 3306 (BR); Kiluluma, Walikale Territory, A. Léonard 2063 (BM).
Kasai-Katanga: Kanda Kanda-Kaniama Territories, Societé Sacomintra, Quarré 2194 (BR).
Katanga: Tshibili R., west of Kolwezi, Schmitz 3073 (BR).

Angola: Congo Iala, Zaire District, Gossweiler 8636, p.p. (LISU); Sumba, Peco, Congo District, near Zaire R., Gossweiler 8680 (BM, K); between Machinge and and Malinda(?), Luachimo R., Marques 267 (COI, LISU); Golũngo Alto, Welwitsch 6021 (BM, 5 sheets, with juv. spec., C, COI, G, K, LISU, 8 sheets, with juv. spec., P, lectotype of *A. macrantha*); Pũngo Andongo, Welwitsch 6022 (BM, C, COI, G, K, LISU, 3 sheets, P, paratype of *A. macrantha*).

N. Rhodesia: Matonchi R., below dam, Mwinilunga District, Milne-Redhead 2846 (BM, BR, K, with fl. buds in spirit coll.); Zambesi R., north of Kalene Hill, Mwinilunga District, Western Province, Angus 557 (K); near the headwaters of the Lunga R., Mwinilunga-Kolwezi Road, Mwinilunga District, Angus 574 (BM, BR, K).

Tanganyika: west of Bukoba, Bukoba District, Lake Province, Eggeling 6251 (EA, K); Nyakato, Bukoba District, Gillman 265 (EA, K); between Kibondo and Mabamba, Kibondo District, Western Province, Procter 593 (EA, K).

Kenya: Kakamega, North Kavirondo District, Feltham 10290 (EA).

Uganda: Logiri, West Nile, Buganda Province, Eggeling 1858 (F.D. 1660) (K); near Hoima, Bunyoro, Bagshawe 1459 (BM); south of Buddu, Masaka District, Fyffe 14 (K); Mengo District, Dümmer 2970 (BM, K); Ruampara, Rwoho, Ankole District, St. Claire Thompson 1815 (EA, K, MO); near Kampala, Wilson 259 (A).

Sudan: bank of Nabagoon R., Yambio District, Andrews A. 1568 (K).

A. vogelii differs from *A. nobilis* mainly by the comparatively shorter corolla tube. The leaves of *A. nobilis* are usually comparatively narrower and drying black; the fruits are shrivelled when dry. In *A. vogelii* the peduncles and the branches are stouter, and the fruits

not shrivelled when dry. Ecologically they are separated, as *A. vogelii* grows in swamps and *A. nobilis* in dry places.

Some plants of *A. schweinfurthii* may have spines, but can be distinguished from *A. vogelii* by their leaves which are usually petiolate and have no revolute margin. But the most typical feature of *A. schweinfurthii* is the characteristic tapering flower bud.

Remarks: *A. buchneri* falls entirely within the variation of *A. vogelii* according to its description. Partially united filaments occur only in some specimens (Bels 28, Louis 13544, and Marques 267) which belong certainly to *A. vogelii* as shown by all other characters.

A. lanceolata falls also within the variation of *A. vogelii* according to its description, except for its comparatively narrow leaves which are about 6 × as long as wide.

The type of *A. macrantha* has in its flowers the same proportions as *A. vogelii* but the corolla is larger; besides it has incipient spines or unarmed branches. Some other specimens of *A. vogelii* have very short spines, as for instance Ghesquière 4943, Gutzwiller 829, and Onochie FHI 40448, which fall in all other characters within the variation of the species.

The specimen St. Claire Thompson 1815, discussed by Miss BRUCE (1955), has a proportionally long corolla tube which does not fall outside the variation of the species. The same is true for Corbisier-Baland 1235, 1960, and Donis 2401.

6. **A. djalonensis** A. Chev., Bull. Soc. Bot. Fr. 54. Mém. 8: 47. 1908; Bruce, Kew Bull. 1955: 49; Aubréville, Fl. For. Cot. Iv. 2nd. ed. 3: 184. 1959.

Figs. 8-10; Map 6

Lectotype: French Guinea: Kollangui, Chevalier 12221 (P, 2 sheets).

Heterotypic synonym: *A. kerstingii* Gilg ex Volkens, Notizbl. Bot. Gart. Berlin App. 22: 33. 1909; Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 18. 1931, p.p. excl. Kersting A 627; Aubréville, op. cit. 1st. ed. 3: 154. 1936. Type: Togo: Sokode, Kersting A 18 (holotype not seen, destroyed in B; isotypes: E!, K!).

Misapplied names: *A. nobilis* Hutch. et Dalz., l.c., quoad syn. *A. djalonensis* A. Chev., non G. Don.

A. procera Leprieur ex A. Chev., Expl. Bot. 441. 1920, non ex Bureau.

A. procera Leprieur ex A. Chev., l.c., var., non ex Bureau.

Tree, 8–15 m high. Twigs with or without 2 erect or slightly divergent confluent spines or single small cushions above the leaf-axils. Leaves petiolate; blade pale to dark green above, paler and with pale yellowish-green midrib and veins beneath, slightly shining on both sides, coriaceous or in young plants softly subcoriaceous, brittle, when dry greenish-brown and coriaceous to papyraceous, oblong-elliptic or obovate-elliptic, in young plants usually narrower, 1.5–3.5 × as long as wide, 9–35 × 4.5–17 cm, in young plants and low-level branches up to 115 × 50 cm, obliquely cordate, rounded,

or cuneate at the base or decurrent into the petiole; veins not very conspicuous; margin not recurved. Sepals pale green, rounded, the outer ones orbicular, 6–10 mm long, more or less rugulose and not or scarcely spreading when dry, the inner ones of about the same size and shape, becoming retuse by the development of the corolla. Corolla in the young bud at the apex as in the mature bud or slightly tapering, in the mature bud 5.5–6 × as long as the calyx, 30-50 mm long and rounded or subtruncate at the apex, white or creamy, the limb paler than the tube; tube 3–4 × as long as the calyx, 2–3 × as long as the lobes; lobes 11–14, oblong-lanceolate, spreading. Berry ellipsoid, when dry not shrivelled and rounded at the apex.

Trunk cylindric, without buttresses, 15–40 cm in diam.; twigs about 1–2 cm in diam. *Leaves* of a pair subequal or unequal (the larger one up to twice as long as the other); petiole 0.1–0.25 × as long as the blade, auriculate at the base; blade more shining when young, mostly rounded at the apex, acuminate to obtuse in young plants; costa and veins prominent beneath; secondary veins 7–19 pairs. *Inflorescence* 3–4 × branched, dichasial or sometimes by development of descending serial axillary branches partially 4-chasial, about 15–50 cm long and wide. Peduncle, branches, and pedicels greenish-white or pale green and with darker green dots, thickened at the nodes. Upper bracts triangular, about as long as or shorter than the diameter of the corresponding branch, acute. Corolla tube nearly cylindric, 20–32 mm long, contracted at the base when young, gradually widened towards the throat, 4–5 mm wide at the base, 10–12 mm at the throat; lobes 2.5–4 × as long as wide, 10–18 × 4–6 mm, obtuse. Filaments entirely connate; anthers creamy or sometimes buff-yellow, about 4.5 × as long as wide, 7 × 1.5 mm, sagittate at the base. Ovary obovoid, about 2 × as long as wide, 6–7 × 3.5 mm, thick-walled; stigma obovoid-cylindric, about 2 × as long as wide, 5–6 × 2.5–3 mm, apically bilobed. Berry dark green, hard, rounded at the apex, about 1.2–1.7 × as long as wide, 3.5–5 × 2–3.5 cm; wall 3 mm thick; septa about 0.75 mm thick. Seeds medium brown, obliquely ovate or ovoid, 2.5 × 1.5–1.7 × 1 mm.

Distribution: Tropical West Africa, from Portuguese Guinea to Cameroun.

Ecology: In rather dry places, in savannas or thickets. Alt. 0–500 m.

Portuguese Guinea: Teixeira Pinto, Orey 130 (K, LISC); Gabu, Espírito Santo 274 (COI); Piche-Pansor, Espírito Santo 3425 (COI); near Fulacunta, Orey 211 (COI, K).

Guinea: Kollangui, Chevalier 12221 (P, 2 sheets, lectotype; photograph of one sheet in K), 12222 (P, paratype; photograph in K), 12873 (P); Santa Valley, Chevalier 12773 (P); between Santa R. and Timbo, Chevalier 12801 (P, juv.); Kaloum, Conakry, Maclaud 40 (P); Kindia, Chevalier 13390 (P).

Sierra Leone: Musaia, Deighton 4215 (K), 4827 (K, BR, EA).

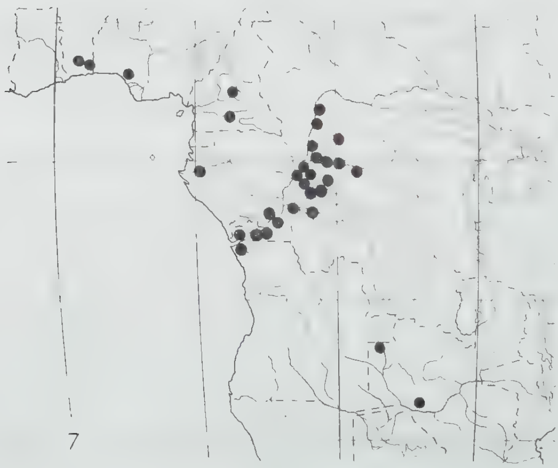
Ivory Coast: near Séguéla, Leeuwenberg 3273 (WAG); 25 km west of Bouaké, Leeuwenberg 3285 (WAG, with fr. in spirit coll.; Photograph 4; seedlings of this number in the greenhouses in WAG, BR, K, L, U); 9 km south of Bouaké, Leeuwenberg 3315 (WAG); 11 km south of Bouaké, Leeuwenberg 3316 (WAG, with fl. and fr. in spirit coll., L); Forêt de l'Angedédou, northwest of Abidjan, Herb. I.D.E.R.T. 3671 (ABI); Adiopodoumé, 17 km west of Abidjan, Ake Assi Herb. I.D.E.R.T. 5412 (P, WAG); Lagune Ono, near Grand Bassam, Chevalier 33055 (P); east of Bingerville, Leeuwenberg 3167 (WAG, Photograph 5); Tafiré-Soba, Aubréville 1393 (P); Ferkessedougou, Aubréville 1538 (P), 2610 (P).

Upper Volta: between Soukourouba and Bobo-Dioulasso, Guiri, Chevalier 886 (P, K).

Ghana: Ejura, Vigne 2029 (BM, EA); Kwahu, between Kwahu and Tafo, Irvinge 1669 (E, K).

Togo: Sokode, Kersting A 18 (E, K, isotypes of *A. kerstingii*); Lenso (?), Kersting A 355 (BO).

DAHOMEY: Adja, Ouéré, Le Testu 296 (P); Godomey, Poisson July 1902 (P).
NIGERIA: near Ibadan, road to Oyo, Meikle 1309 (K, with fl. buds in spirit coll.); Oyo Province, Lapido FHI 3086 (K); ibid., Ibadan Government College, Keay FHI 26734 (K); ibid., Hambler 7 (K); Ibadan, Onochie FHI 38332 (K, WAG); Olokemeji Reserve, Ross 39 (K); between Onitsha and Uke, Onochie FHI 35775 (K); Onitsha Province, Akpaka Forest Reserve, Onochie 40433 (K, with fr. in spirit coll.); Abinsi, Benue Province, Dalziel 638 (K); Bauchi Province, Tangale Waje District, Kennedy FHI 7275 (K); North of Orukram (?), Dundas 1933/21 (A); S. Nigeria, sin. loc., Rosevear 50/29 (K).
CAMEROONS: Jamtari, between Jamtari and Karamti, Latilo & Daramola FHI 28928 (K).
CAMEROUN: sin. loc., Aubréville 166 (P, WAG).



Map 6. *A. djalonensis;* Map 7. *A. liebrechtsiana.*

7. **A. liebrechtsiana** De Wild. et Dur., Compt. Rend. Soc. Bot. Belg. 38(2): 96. 1899; Baker in Fl. Trop. Afr. 4(1): 540. 1903.
Fig. 11; Map 7

Type: Congo: Equateur, Lukolela, Dewèvre 829 (BR, 2 sheets; photographs of both in K).

Heterotypic synonyms: *A. baertsiana* De Wild. et Dur., Bull. Herb.

Boiss. Sér. 2. 1: 829. 1901; Baker, l.c. p. 625. 1904. Type: Congo: Leopoldville, Kisantu, Gillet 56 (BR, 9 sheets; photographs of 2 sheets in K).

A. gossweileri Exell, Journ. Bot. 67. Suppl. 2: 101. 1929. Type: Angola: Zaire District, Congo Iala, Gossweiler 8636, p.p. (BM, holotype; isotypes: BM, K; the LISU sheet is *A. vogelii*).

Tree or few-stemmed (?) shrub, 1.50–12 m high, without spines. Leaves petiolate; blade dark green above, pale greyish-green beneath, drying greenish-brown and coriaceous, oblanceolate to linear or sometimes oblong-obovate, 2½–10 (usually about 4–7) × as long as wide, 11–75 × 3–15 (usually about 15–40 × 3–8) cm, long-decurrent into the petiole; costa prominent and acutely triangular beneath; secondary veins rather inconspicuous; margin not recurved nor revolute. Sepals pale green, rounded, also when dry strongly appressed to the base of the corolla tube which therefore is slightly contracted and later to the fruit, smooth and shining, the outer ones broadly ovate or orbicular, 4–8 × 4–8 mm, the inner ones slightly larger and becoming torn by the development of the corolla. Corolla in the mature bud 4½–7 × as long as the calyx, 32–54 mm long, and as in the young bud rounded or somewhat tapering at the apex, white, the limb paler than the tube which is usually greenish-white; tube 3–5 × as long as the calyx, 1.8–3 × as long as the lobes; lobes 10–12, oblong-lanceolate, spreading. Berry globose or ovoid, when dry irregularly shrivelled, thin-walled; wall about 1 mm thick.

Trunk 10–30 cm in diam. or more(?), without buttresses(?); twigs about 0.5–1.5 cm in diam. *Leaves* of a pair subequal; petiole $^1/_{15}$–$^1/_5$ × as long as the blade, up to 9 cm long, auriculate, obscurely ligulate at the base; blade mostly rounded at the apex, acuminate to obtuse in young plants; secondary veins 7–19 pairs. *Inflorescence* 3–5 × branched, 10–35 cm long and wide. Peduncle, branches, and pedicels not or scarcely thickened at the nodes. Upper bracts triangular, about as long as wide, about 1–2 × as long as the diameter of the corresponding branch, obtuse. Outer sepals 0.8–1.2 × as long as wide. Corolla tube narrowly infundibuliform, 22–34 mm long, contracted at the base, especially when young, gradually widened towards the throat, 3–5 mm wide at the base, 6–10 mm at the throat; lobes 2½–3 × as long as wide, 10–18 × 4–6 mm, obtuse. Filaments entirely connate; anthers 4–5½ × as long as wide, 7–8 × 1.5–2 mm, sagittate at the base. Ovary obovoid, about 2 × as long as wide, 5–7 × 3–4 mm, with a rather thick wall; stigma obovoid-cylindric, about 2 × as long as wide, 3.5–5 × 2–2.5 × 1.5–2 mm, emarginate at the apex. Berry green or pale yellow, 1–1½ × as long as wide, 15–27 × 10–18 mm, rounded at the apex, when dry often acuminate, dull. Seeds medium brown, obliquely ovate or roughly polyhedral, 1½–2 × as long as wide, 1.5–2.5 × 1–1.5 mm. Embryo about 1.5 × 0.2 mm.

Distribution: From Togo to Angola and in Northern Rhodesia.

Ecology: In open places in swamps or in the water, in usually periodically inundated forests. Alt. 0–400 m.

DAHOMEY: between Massi and Goutyssa, Abomey District, Chevalier 23269 (P); banks of Lower Ouémé R., between Dogba and Affamé, Chevalier 23472 (P).

NIGERIA: Siluko, bank of Siluko R., Benin District, Onochie FHI 40423 (K, with fl. and fr. in spirit coll., WAG).

CAMEROUN: Ayos, Jaques Félix 4862 (P, immature fruits only); S. Kongola, Mildbraed 9001 (K).

GABON: near Atsié R., near Lambaréné, Chevalier (Fleury) 26305 (P); near Brazzaville, Pobéguin 2 (P); ibid., J. de Brazza 136 (P).

CONGO: LEOPOLDVILLE: Kiri R., near Lake Léopold II, Laurent 5 Nov. 1903 (BR); Lake Léopold II, Jans 249 (BR); ibid., Bokebeni, Flamigni 6395 (BR, with juv. spec.); Ebu R., Banningville Territory, Jans 910 (BR); Lower Kasai R., Bokala, Vanderyst 4785 (BM, BR); Léopoldville, Bequaert 7312 (BR); ibid., Gillet 2543 (BR); Kisantu, Gillet 56 (BR, 9 sheets, type of *A. baertsiana*); Kitobola (= Kito), southeast of Thysville, Flamigni 147 (BR); Zambi, mouth of Congo R., Bequaert 7904 (BR).

EQUATEUR: between Libenge and Dongo, near Ubangi R., Lebrun 1672 (BR); Bombura, Lua-Vindu R., Evrard 936 (BR); Bomana, Giri R., Sapin anno 1912 (BR); Ukatoraka, Laurent 22 Jan. 1904 (BR, photograph in K); Eala, Coquilhatville Territory, Corbisier-Baland 1815 (BR, WAG); ibid., Evrard 3698 (BR, WAG); ibid., Germain 1603 (BR); ibid., Leemans 219 (BR), 280 (BR), 382 (BR); ibid., Pynaert 548 (BR); ibid., Vermoesen 2352 (BR, PR); near Eala, Yali R., Robijns 550 (BR); Ruki R., Eala, Coûteaux 31 (BR), 128 (BR, K); ibid., Lebrun 378 (BR); ibid., J. Léonard 182 (BR, WAG); Bonkele R., between Bamania and Ilenge, near Eala, J. Léonard 971 (BR); Bikoro, Evrard 3751 (BR, WAG); Bamania, Hulstaert 863 (BR); Bokuma, left bank Ruki R., Hulstaert 183 (BR); between Bantoie and Bojeka, left bank Ruki R., Louis 1893 (BR, K); Bokote, Busira R., Hulstaert 329 (BR), 822 (BR); Gombé, Sapin anno 1912 (BR); Lukolela, Dewèvre 829 (BR, 2 sheets, type); Elua Island, Lake Tumba, J. Léonard 675 (BR); Bikoro, between Lake Tumba and Mabali, Thonnet 52 (BR); Boleke, Bolamba Territory, L. Dubois 305 (BR, WAG); between Kutu and Lukolela, Lebrun 6622 (BR, K).

CONGO: sin. loc., Greshoff 2 (L).

ANGOLA: Congo Iala, near Congo R., Gossweiler 8636, p.p. (BM, 2 sheets, K, type of *A. gossweileri*).

N. RHODESIA: north of Chavuma, Balovale District, Western Province, Angus 642 (BM, BR, K), 642A (K); Katombora, Southern Livingstone, Brenan & Morze 7740 (EA, K).

A. liebrechtsiana has narrow leaves, drying pale greenish-brown, and the very small calyx by which it can be distinguished from *A. schweinfurthii*.

From *A. djalonensis* it can be separated also by its narrow leaves, the blade never being cordate at the base, and the small calyx clasping the corolla also when dry. Moreover *A. liebrechtsiana* has never spines and grows in moist places, and its fruits have a thin wall.

8. **A. schweinfurthii** Gilg in Engl. Bot. Jahrb. 17: 579. 1893; Baker in Fl. Trop. Afr. 4(1): 541. 1903; Bruce, Kew Bull. 1955: 51; Bruce & Lewis in Fl. Trop. E. Afr., Loganiaceae 11. 1960.

Fig. 12; Map 8

Lectotype: Congo: Orientale, Niamniam, Brwole R., Niangara Territory, Schweinfurth 3726 (K, 4 sheets, lectotype; isotypes: S, WU).

Heterotypic synonyms: *A. niamniamensis* Gilg, l.c. p. 580. Type: Sudan: Niamniam, Nabambisso R., Boddo R., Schweinfurth 3037 (holotype not seen, destroyed in B; isotype: K!).

? *A. stuhlmannii* Gilg, l.c. p. 580; Baker, l.c. p. 540. Type: Tanganyika: Lake Province, Bukoba, Stuhlmann 3737 (holotype not seen, destroyed in B; no isotype seen). Topotype: Eggeling 6237 (EA, K, = *A. schweinfurthii*!).

A. magnifica Gilg, l.c. p. 581; Baker, l.c. p. 541. Type: Gabon: Munda, near Libreville, Sibange Farm, Soyaux 49 (holotype not seen, destroyed in B; isotypes: GOET!, K!, P!, Z, 2 sheets!).

? *A. kamerunensis* Gilg in op. cit. 28: 125. 1899; Baker, l.c. p. 538. Type: Cameroun: Batanga, Dinklage 1361 (holotype not seen, destroyed in B; no isotype seen).

A. squamata De Wild. et Dur., Bull. Herb. Boiss. Sér. 2. 1: 830. 1901; Baker, l.c. p.625. 1904. Type: Congo: Leopoldville, Kimuenza, Gillet 1773(BR, holotype; photographs: EA, K).

Map 8. *A. schweinfurthii.*

Map 9. *A. grandiflora.*

A. insulana S. Moore, Journ. Linn. Soc. 37: 186. 1905. Type: Uganda: Buganda Province, Mengo District, Lake Victoria, Buvuma Island, Bagshawe 638(BM, holotype).

A. laurentii De Wild., Miss E. Laurent 1: 262. 1906. Type: Congo: Equateur, near Lukolela, Em. & M. Laurent 13 Dec. 1903 (BR, holotype, corollas are *A. schweinfurthii*!; leaves may be *A. liebrechtsiana*).

A. pynaertii De Wild., Ann. Mus. Congo Sér. 5. 3: 251. 1910. Type: Congo: Equateur, near Coquilhatville, Eala, Pynaert 855 (BR, 2 sheets; photograph of one sheet in K).

? *A. gigantea* Gilg in Mildbraed, Wiss. Erg. Deutsch. Centr. Afr. Exp. 1907–8. 2: 533. 1913. Type: Ruanda: Lake Kivu, Wau Island, Mildbraed 1140 (holotype not seen, destroyed in B; no isotype seen).

A. oubanguiensis Aubrév. et Pellegr., Bull. Soc. Bot. Fr. 100: 25. 1935; Sillans, Les Savanes Afr. Centr. 330, f. 70. 1958. Type: Oubangui-Chari: Ouaka District, 25 km southwest of Ippy, Tisserant 1934 (P, holotype; isotypes: IFAN, P; photograph of one P sheet in K).

Misapplied name: *A. nobilis* Baker, l.c. p. 539, quoad spec. Schweinfurth 3037 et 3726, non G. Don.

Tree 3–30 (usually 4–20) m high. Twigs without or occasionally, especially in young plants, with short paired partially united spines, often with small broadly conical cushions. Leaves usually petiolate but often sessile in young plants or in low-level branches; blade dark green and usually (?) glossy above, paler beneath, when dry medium to dark brown above, paler beneath, papyraceous to coriaceous, oblong-obovate to oblanceolate, in young plants usually narrower, 1.75–3.5 × as long as wide, 7–45 × 3.5–18 cm, in young plants up to 100 × 30 cm or more (?), cuneate at the base; costa more or less acute beneath; tertiary veins inconspicuous; margin not recurved, but often revolute. Sepals green, rounded, when dry usually smooth, especially the outer ones, in flower usually appressed to the base of the corolla, in fruit often spreading, the outer ones orbicular or slightly broader than long, 8–13 × 9–13 mm, the inner ones usually slightly larger, becoming retuse by the development of the corolla. Corolla in the mature bud 5½–7 × as long as the calyx, 55–61 mm long, tapering at the apex as in the young bud, white or creamy, tube darker than the lobes, often greenish-white; tube about 3–4 × as long as the calyx, about 1–1½ × as long as the lobes; lobes 10–11, lanceolate, reflexed. Berry globose or ellipsoid, rounded or apiculate at the apex, never shrivelled when dry; wall thick.

Trunk 8–70 cm in diam., without buttresses; twigs about 1–2 cm in diam. *Leaves* of a pair subequal; petiole up to ¼ × as long as the blade, auriculate at the base or often exauriculate in higher leaves; blade when young shining on both sides, mostly rounded at the apex, sometimes acute, obtuse, retuse, or shortly apiculate, more acute in young plants, entire or minutely crenate; costa and secondary veins prominent beneath; secondary veins 6–17 pairs. *Inflorescence* 4 × branched, 12–30 cm or more (?) long and wide. Peduncle, branches, and pedicels thickened at the nodes. Upper bracts about 2–6 × as long as the diameter of the corresponding branch, ovate or oblong-ovate, about 1–2 × as long as wide, 4–9 × 3–5 mm, rounded. Corolla tube nearly cylindric, 25–37 mm long, contracted at the base when young, gradually widened towards the throat, 6–8 mm wide at the base, 12–14 mm at the throat; lobes 3–4½ × as long as wide, 21–28 × 5–8 mm, obtuse. Filaments entirely connate; anthers about 5 × as long as wide, 7.5–10 × 1.5–2 mm, sagittate at the base. Ovary ovoid or nearly so, about

7 × 3.5 mm, thick-walled; stigma obovoid-cylindric, about as long as wide, 3–5 × 3–4 mm, apically emarginate. Berry green or yellow, hard, 1–1.2(1.5) × as long as wide, 25–45 × 20–30 mm, up to at least 70 mm long (*teste* Dawkins 846). Seeds medium brown, obliquely ovate-orbicular, about 2 × 1.5 mm.

Distribution: Central Africa, from Nigeria to the Sudan in the North and Angola and Tanganyika in the South.

Ecology: In secondary or gallery forests, in thickets, or sometimes in savannas or rain forests, usually not in moist places. Alt. 400–1800m, or less (?).

NIGERIA: Sapoba, Jamieson R., Benin District, Kennedy 2136 (K, P).

CAMEROONS: Johan Albrechtshöhe, Büsgen 15 (B).

OUBANGUI-CHARI: Ouadda, Aubréville 387 (P); Boukoko, north of Bangui, Tisserant 1037 (P), 1800 (P); 25 km southeast of Ippy, Ngukpwanga R., near Ouaka R., Tisserant 1934 (IFAN, P, 2 sheets, type of *A. oubanguiensis*); Upper Kourou R., Chevalier 7367 (P), 7376 (P); Obo, Haut M'Bomou, Aubréville 542 (P).

CAMEROUN: near Douala, Chevalier (Fleury) 33292 (P, WAG), ibid., Winkler 737 (Z); between Bipindi and Ebolowa, Mildbraed 7591 (K); Coupe Malo, near Bipindi, Hedin 1533 (P, WAG).

GABON: Sibang, near Libreville, Soyaux 49 (GOET, K, P, Z, 2 sheets, isotypes of *A. magnifica*); near Lastourville, Le Testu 7737 (P).

MOYEN CONGO: Lower Kouilou R., Sargos 20 (P).

CONGO: LEOPOLDVILLE: Inongo, Lake Léopold II, Gilbert 14147 (K); Kimuenza, Gillet 1773 (BR, type of *A. squamata*); ibid., Carlier 304 (BR, K, M); ibid. (?), Gillet (Butaye) 1831 (BR); banks of Lukaya R., Gillet (Gérard) Jan. 1900 (BR); Kisantu, Callens 2981 (BR); Mvuazi, Thysville Territory, J. Dubois 28 (BR, with fl. in spirit coll.); ibid., Devred 300 (BR, K); Seke Banza-Gimbi Road, Compère 193 (BR); Luki R., Madoux 53 (BR, K), 223 (BR, with fl. in spirit coll., WAG); ibid., Toussaint 2011 (BR); Ludio R., left bank Kwango R., Germain 2728 (BR, P); between Mazia and M'Pata, Dibaya Territory, Liben 3216 (BR); Lutshima, Sapin July 1907 (BR).

EQUATEUR: Bolombo, north of Coquilhatville, Laurent 2 Jan. 1904 (BR); Eala, near Coquilhatville, Pynaert 855 (BR, 2 sheets, type of *A. pynaertii*); Mondjo, Ikelemba, J. Léonard 510 (BR); km 10, Bikoro-Inongo Road, Evrard 2664 (BR); Mondombe, Evrard 5513 (BR, WAG); Isandja, Evrard 2853 (BR, WAG); near Lukolela, Laurent 13 Dec. 1903 (BR, type of *A. laurentii*); near Busira R. (?), Hulstaert 1369 (BR).

ORIENTALE: Tukpwo, Ango Territory, Gilbert 417 (BR, WAG); ibid., Gérard 1200 (K); Kumbu, near Doruma R., De Graer 594 (BR); Brwole R., Niangara Territory, Schweinfurth 3726 (K, 4 sheets, S, WU, lectotype); Bambesa, Gérard 203 (BR), 3844 (BR); between Boruma and Niangara, Lebrun 3186 (BR, WAG); Madili, near Doruma, Dungu Territory, Leclercq 141 (BR); Yambao, north of Yangambi, Gilbert 1399 (BR); Weko, north of Yangambi, Louis 14069 (BR); Yangambi, Louis 16425 (BR); ibid., near Lusambila R., Louis 3100 (BR, K), 9521 (BR); near Busukuru, near Yangambi, Louis 7873 (BR, K); Yalibwa, northwest of Yangambi, Louis 10017 (BR, WAG); Yawenda, near Yangambi, Gutzwiller 566 (BR); Ituri Forest, Epuhi, between Penghe and Irumu, Bequaert 2598 (BR).

KIVU: Rutshuru, Ghesquière 3691 (A, BR, K, MO, P, U); Bitale, km 48 Kavumu-Walikale Road, Pierlot 603 (BR), 1531 (BR); Kasheke, Kalehe Territory, A. Léonard 1249 (BM, BR, WAG); Makengere, Kalehe Territory, near Kivu Lake, A. Léonard 4601 (BR, WAG); Idjwi, Kivu Lake, Michelson 307 (BR); Mulanga, km 128 Bukavu-Shabunda Road, Pierlot 2451 (BR), 2541 (BR, WAG); Kabumba, Lumana-Nyangwe Road, Kasongo Territory, Germain 7704 (BR).

KASAI: Okoka, T. Lodja-Sankuru, Germain 7578 (BR, with fl. in spirit coll., K); Miambi Road, Bakwanga Territory, Liben 1946 (BR).

KATANGA: Kamunza, Schmitz 5763 (BR); Elisabethville, Salèsiens S. 1061 (BR); ibid., Burtt-Davy 17972 (BM, K); ibid., Schmitz 2294 (BR); Keyberg, southeast of Elisabethville, Schmitz 2842 (BR, K), 3332 (BR); Katuba, southeast of Elisabethville, Quarré 4301 (BR, K); sin. loc.: Ringoet 30 Nov. 1916 (K); Delvaux 695 (Letieshe 164) (BR).

CONGO: sin. loc., Chr. Smith s.n. (BM, K).

ANGOLA: Porto Amboim, Capir, Gossweiler 10070 (BM, K, both corollas only); Cuaza Sul District, Gossweiler 5984 (BM, COI, LISU, aberrant); between Vila Henrique de Carvalhoe and Muriége, Lunda, Exell & Mendonça 791 (BM, COI).

N. RHODESIA: near Samfya, Lake Bangweulu, Fort Roseberry District, Angus 276 (BR, K, with fr. in spirit coll.), 291 (BM, BR, K, with fr. in spirit coll.): Zambesi R., north of Kalene Hill, Mwinilunga District, Angus 557A (K): Ndola District, Fanshawe 1684 (BR, K), 2308 (K, young seedling); sin. loc., Holmes 899 (K, with fr. in spirit coll.).

TANGANYIKA: Rubare, Bukoba, Bukoba District, Eggeling 6237 (EA, K, topotype of *A. stuhlmannii*); ibid., Procter 806 (EA, K); Ukara Island, Lake Province, A. Smith 1 (EA); Mahali Mts., Kigoma District, Jefford & Newbould 2602 (K); Malagarasi Pontoon Camp, Kibondo Road, Buha District, Bullock 3223 (K).

UGANDA: Bwamba, Toro District, Eggeling 4061 (K); Entebbe, Dawe 950 (K, P); Mengo District, Buvuma Island, Bagshawe 638 (BM, holotype of *A. insulana*); Chuzezi (?), Bagshawe 122 (BM); Katera, Masaka District, Drummond & Hemsley 4504 (BR, EA, FI, K, with fr. in spirit coll., S); Kalangala, Bugula Island, Buganda Province, Philip 433 (EA, MO); near Sozi Point, Sese Island, Masaka District, Eggeling 89 (F.D. 262) (BR, EA, K); ibid., Maitland 643 (K); Nkose Island, Lake Victoria, Buganda Province, Dawkins 846 (BM, EA, K, fl. and fr. in spirit coll.), 883 (BM, EA, K).

SUDAN: Niamniam, Nabambisso R., Boddo R., Schweinfurth 3037 (K, isotype of *A. niamniamensis*); Aba, Aloma Plateau, Yei District, Meyers 10228 (K); Lado, Yei R., Sillitoe 307 (K, P).

Remark: After comparison of the specimens studied by Miss BRUCE (1955) and many others, several of which had been collected after her publication, her conclusions about synonymy could be confirmed. *A. gigantea* and *A. kamerunensis* fall also within the variation of this species, judging from their descriptions.

9. **A. grandiflora** Gilg in Engl. Bot. Jahrb. 17: 582. 1893.
Fig. 13 (8-13); Map 9

Type: Comores: Corani Region, Schmidt 240 (holotype not seen, destroyed in B; no isotype seen). Neotype: Comores: sin. loc., Humblot 311 (P, neotype; isoneotypes: BM, K, P, 5 sheets).

Heterotypic synonyms: *A. zambesiaca* Bak., Kew Bull. 1895: 99 (April 1895); Fl. Trop. Afr. 4(1): 540. 1903; Prain & Cummins in Fl. Cap. 4(1): 1049. 1909; Bruce, Kew Bull. 1955: 54; Bruce & Lewis in Fl. Trop. E. Afr., Loganiaceae 10, f. 2. 1960. Type: Nyasaland: Shire Highlands, Buchanan 84 (K, holotype; isotypes: E, K).

A. insignis Galpin, Kew Bull. 1895: 150 (June–July 1895). Type: Swaziland: Horo Forest, Leyson 1358 (K, 6 sheets, holotype; isotype: BOL, 3 sheets).

A. orientalis Gilg in Engler, Pfl. Ost-Afr. C: 312. 1895 (Aug. 1895); Baker in Fl. Trop. Afr. 4(1): 539. 1903; Engler & Drude, Veg. der Erde 9(1): 296. f. 262. 1910. Type: Tanganyika: Eastern Province, Uzaramo, Vikindu, Stuhlmann 6092 (holotype not seen, destroyed in B; no isotype seen).

A. scheffleri Gilg ex Scheffler, Notizbl. Bot. Gart. Berlin 3: 143, 144. 1901 (21 Oct. 1901) (with description of living plant); Baker, l.c. p. 542. Type: Tanganyika: Tanga Province, Usambara Mts., Nguelo, Scheffler 55 (holotype not seen, destroyed in B; isotype: E!).

A. pulcherrima Gilg in Engl. Bot. Jahrb. 30: 375, t. 17. 1901 (19 Nov. 1901); Baker, l.c. p. 540. Type: Tanganyika: Southern

Highlands Province, Rungwe District, Umuaba and Yunguru Crater Lake, Goetze 1313 (holotype not seen, destroyed in B; no isotype seen).

A. keniensis Summerhayes, Kew Bull. 1926: 244. Type: Kenya: Nyanza Province, Kericho District, Sotik, Battiscombe 1301 (K, holotype; isotypes: EA, 2 sheets, K, 2 sheets).

Tree, 5–35 m high, unarmed. Leaves sessile, those of larger trees sometimes petiolate; blade medium to dark green above, paler beneath, when dry greenish, medium to dark brown, paler beneath, brittle, often thinly papyraceous to coriaceous, oblong-obovate or oblanceolate, in young plants usually narrower, 1.75–3.5 (in young plants up to 5) × as long as wide, 15–70 × 7–25 cm, up to 135 × 50 cm in young plants or low-level branches, narrowed to the auricles or long-decurrent into the petiole; veins conspicuous; margin not recurved. Sepals green, rounded, usually spreading when dry, the outer ones orbicular or broadly ovate, 5–8 × 5–8 mm (up to 14 × 9 mm in Dawe 472), when dry rugulose outside and often pointed, the inner ones usually slightly larger. Corolla in the young bud rounded or tapering, in the mature bud 5–10 × as long as the calyx, 35–60(70) mm long, tapering at the apex, white, the limb paler than the tube which is slightly greenish outside; tube 3.8 6.5 × as long as the calyx, 1.25–2.5 × as long as the lobes; lobes 11–13, lanceolate, spreading or recurved. Berry ellipsoid, when dry irregularly shrivelled, conspicuously rugulose, acuminate.

Trunk 25–55 cm in diam.; twigs about 1–2 cm in diam. *Leaves* of a pair subequal or unequal; petiole—if present—up to $^{1}/_{6}$ × as long as the blade, auriculate at the base; blade with yellow midrib and veins on both sides (*teste* Maas Geesteranus 5751), mostly rounded at the apex, more acute in young plants, entire or minutely crenate; costa rounded, and like the veins prominent beneath; secondary veins 8–17 pairs. *Inflorescence* 3–5 × branched, 15–45 cm long and wide. Peduncle, branches, and pedicels thickened at the nodes. Upper bracts broadly triangular, about 1–2 × as long as the diameter of the corresponding branch, acute or rounded. Outer sepals 0.75–1.25 × as long as wide. Corolla tube nearly cylindric, 22–37(45) mm long, contracted at the base when young, almost gradually widened towards the throat, 3–7(10) mm wide at the base, 6–12 mm at the throat; lobes 2.2–4 × as long as wide, 13–22 × 5–10 mm, obtuse. Filaments entirely connate; anthers buff (*testibus* Drummond & Hemsley 3020), about 5 × as long as wide, 7–10 × 1.5–2 mm, sagittate at the base. Ovary ovoid- or obovoid-cylindric, about 2–2.7 × as long as wide, 7–9 × 3–3.5 mm, thick-walled; stigma globose or nearly so, about as long as wide, 2–4 mm long, obscurely retuse at the apex. Berry green, 1.5–1.9 × as long as wide, 30–42 × 17–26 mm, rounded at the apex, smooth, when dry entirely changed (see above). Seeds dark brown, obliquely ovate-orbicular, about 1–1.5 × as long as wide, about 2.5 × 1.5–2.5 mm.

Distribution: East Africa, from Uganda and Kenya to Transvaal, and in Zanzibar and on the Comores.

Ecology: In open often swampy places, in rain forests, or in gallery forests, mostly in the mountains. Alt. 0–2300 m.

Congo: Kivu: Ruwenzori Mts., Muboka Valley, Lebrun 4421 (BR, K); Goma, Mushari-Est, Rubona Hills, Spitaels 455 (BR); Rushekero-Katyazo, Mushari, Spitaels 648 (BR); Biwito (= Bwito), Rutshuru Territory, Deru 458 (BR); between Walikale and Kalehe, Lebrun 5374 (BR); Kirumbu-Muero Road, Masisi Territory, Gutzwiller 1944 (BR); Biega, near Masisi, Hendrikx 4098 (BR, K); Idjwi Island,

Lake Kivu, Loveridge 534 (K); Kalwazi Mt., Kabare Territory, near Lake Kivu, Pierlot 379 (BR).
KATANGA: P. N. Upemba, Robijns 3599 (BR); Mwango Plateau, Marungu Mts., Van den Brande 181 (BR).

RUANDA–URUNDI: km 104 Astrida-Shangugu Road, Shangugu Territory, Reynders 308 (BR).

S. RHODESIA: Inyanga, near Hyamingura R., Phipps 1250 (BR, EA); Pungwe R., southeast of Inyangani, Chase 830 (BM); Bikita District, Wild 4379 (K, MO); Chirinda Forest, Chase 379 (BM).

MOÇAMBIQUE: between Mueda and Chomba, Niassa District, Gomes Pedro 5352 (LISU); Gurué, Zambézia District, Campos Andrada 1842 (COI, LISC); ibid., Medonça 2196 (LISC); Massingire, Morumbala Mts., Zambézia District, Torre 4548 (LISC); Báruè, Choa Mts., Vila Gouveia, Manica E Sofala District, Mendonça 287 (LISC); Chimoio, Manica E Sofala District, Simão 526 (LISC); Marongo, Manica E Sofala District, Simão 412 (LISC); Dombe, between Dombe and Sanguéne, Manica E Sofala District, Gomes Pedro 4490 (K); Cheringoma Manica E Sofala District, Mendonça 4450 (LISC); ibid., Durundi Mts., Torre 3080 (LISC), 4202 (LISC); Chiniziua, near Chengo R., near Beira, Gomes E Sousa 4366 (K); Mufucia Forest, Dawe 472 (K); Maruma Mt., Sul Do Save District, Swynnerton 27 (BM, K); sin. loc., Gomes E Sousa 4260 (BR, EA, K).

NYASALAND: Nchisi, Kota-kota District, Brass 17072 (BM, BR, EA, K, MO); Zomba, Clements 285 (A), 317 (K); Shire Highlands, Buchanan 84 (E, K, type of *A. zambesiaca*); Cholo Mt., Cholo District, Brass 17854 (BR, K, MO); Nelungusi R., Johnston 5 (K).

ZANZIBAR: Tozani Forest, Vaughan 997 (EA, K); New Forest, Burtt Davy 22641 (A).

TANGANYIKA: Nguelo, Usambara Mts., Scheffler 55 (E, isotype of *A. scheffleri*); E. Usambara Mts., Drummond & Hemsley 3020 (K, with fl. in spirit coll.), 3458 (K, with fl. and fr. in spirit coll.); ibid., Peter 3474 (B), 13898 (B), 23265 (B, juv.); ibid., Verdcourt 225 (EA, K, MO); ibid., near Amani, Peter 3751a (B), 21498 (B); ibid., Braun 1082 (EA, FI); ibid., Bryce 51 (EA); ibid., Tanner 3001 (BR, K); Kombola, Unguru Mts., Schlieben 4099 (B, K, LISC, MO); Mtibwa Forest Reserve, Morogoro District, Semsei 1510 (EA, K); Mgeta R., Morogoro District, Drummond & Hemsley 1672 (BR, EA, K, LISC, S); Mahenge, Eastern Province, Schlieben 1991 (BR, G, Z); Kyimbila, Rungwe District, Stolz 497 (B, C, G, HBG, K, L, LD, M, S, U, WAG, Z); between Tukuyu and Kiwera, Southern Highlands Province, Davies 895 (EA, K).

KENYA: near Mutonga R., Meru/Embu District, Fries 1902 (K, S); Kericho District, Battiscombe 698 (EA, K, paratype of *A. keniensis*), 1301 (EA, K, type of *A. keniensis*); Kericho District, Southwestern Mau Forest Reserve, Maas Geesteranus 5751 (BR, COI, K, L, MO, S).

UGANDA: Ruwenzori Mts., Mubuku Valley, Eggeling 1257 (EA); Mt. Elgon, Dale U 78 (BR, K); ibid., St. Claire Thompson E. 3945 (K); ibid., Bulago, Snowden 517 (BM, K, MO).

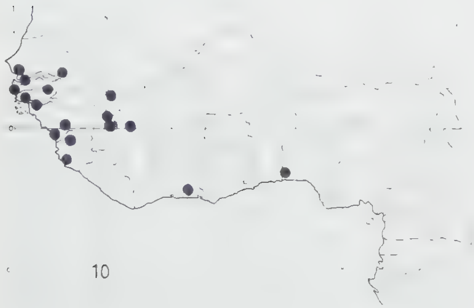
TRANSVAAL: The Downs, Pietersburg District, Moss & Rogers 248 (K, S, Z); between Sibasa and Zoutpansberg, Rodin 4105 (K, MO, S); Zoutpansberg, Brady 17 Oct. 1905 (BM); Burtt Davy 163 (BOL); ibid., Kruger National Park, Punda Maria, v. d. Schyff 1025 (K); Debegeni Fall, De Hoek, Kräusel 15 (M).

SWAZILAND: Horo Forest, Leyson 1358 (K, 6 sheets, BOL, 3 sheets type of *A. insignis*).

COMORES: ANJOUAN: Boivin May 1850 (P).
MOHELI: Kirk 340 (K).
Sin. loc.: Humblot 311 (BM, K, P, 6 sheets, neotype).

Remark: Humblot 311 which has been collected near the type locality of the species agrees in all details, also in the calyx, with the type of *A. zambesiaca*. In many specimens of this species the sepals are conspicuously rugulose and spreading when dry, but not in Buchanan 84, the type of *A. zambesiaca*, Humblot 311, and some other specimens of this species. As the latter has been collected near the type locality it has been selected as neotype.

10. **A. procera** Leprieur ex Bureau, Thèse Logan. 15, 74–77, f. 60–62. 1856; Baker in Fl. Trop. Afr. 4(1): 539. 1903; Bruce, Kew Bull. 1955: 56; Aubréville, Fl. For. Cot. Iv. 2nd. ed. 3: 184. 1959. **Fig. 13 (1-7); Map 10**



Map 10. *A. procera;* Map 11. *A. microphylla;* Map 12. *A. obanensis.*

Type: Gambia: near Albreda, Leprieur 5 June 1827 (P, holotype; isotypes: G, 4 sheets, P, 2 sheets), not Heudelot 109 which had been annoted neither by Leprieur nor by Bureau.

Heterotypic synonym: *A. frezoulsii* A. Chev., Bull. Soc. Bot. Fr. 54. Mém. 8: 47. 1908; Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 18. 1931; Aubréville, op. cit. 1st. ed. 3: 154. 1936. Type: Guinea: Conakry, Chevalier 12162 (P, holotype).

Misapplied name: *A. nobilis* Bak., l.c. p. 538, quoad spec. Leprieur 5 June 1827, non G. Don.

Tree, 6–20 m high, without spines. Leaves sessile; blade medium green above, paler beneath, hardly shining, drying greenish-brown, coriaceous, brittle, when dry coriaceous to papyraceous, oblong-obovate or oblanceolate, 2–2½ × as long as wide, about 40–45 × 20 cm, in young plants and low-level branches up to 145 × 45 cm, gradually narrowed to the auricles, veins conspicuous; margin not revolute. Sepals pale green, rounded, the outer ones rugulose outside and often somewhat spreading when dry, orbicular or broadly ovate,

9–10 × 7–8 mm, the inner ones wider, becoming retuse by the development of the corolla. Corolla in the young bud at the apex as in the mature bud or rounded, in the mature bud 4½–6½ × as long as the calyx, 45–65 mm long, tapering and acute to obtuse, white, slender; tube 3½–5½ × as long as the calyx, 3½ × as long as the lobes; lobes 9 or more (?), oblong, spreading. Berry ellipsoid, rounded, when dry often with 4 regular dents above the septa and obtuse at the apex; wall thick, thin in immature fruits.

Trunk cylindric, without buttresses, 15–50 cm in diam.; twigs about 2–4 cm in diam. *Leaves* of a pair subequal or equal, auriculate at the base; blade when young soft, pale green and shining on both sides, mostly rounded at the apex, entire or minutely crenate; costa and veins on both sides paler, prominent beneath; secondary veins 8–15 pairs. *Inflorescence* 3–5 × branched, about 30–60 cm long and wide. Peduncle, branches, and pedicels pale green, thickened at the nodes. Upper bracts triangular, about as long as the diameter of the corresponding branch or somewhat longer, acute. Corolla tube nearly cylindric, 35–55 mm long, slightly contracted at the base when young, gradually widened towards the throat, 4.5–5.5 mm wide at the base, 8 mm at the throat; lobes 2–3 × as long as wide, 10–15 × 5–6 mm, obtuse. Filaments entirely connate; anthers about 5 × as long as wide, 7 × ca. 1.5 mm. Ovary obovoid to cylindric, 7 × 3.5 mm, thick-walled; stigma obovoid-cylindric, about as long as wide, 2–2.5 × 2–2.5 mm, apically retuse. Berry pale green, shining, hard, 1.25–1.5 × as long as wide, 28–30 × 17–22 mm or more (?). Seeds dark brown, obliquely ovoid-orbicular, 1.25–1.75 × as long as wide, 1.7–2 × 1–1.5 mm.

Distribution: Tropical West Africa, from Sénégal to Nigeria.
Ecology: In open usually swampy places. At low elevations.

SÉNÉGAL: Koular, Berhaut 891 (P, BR); Camance, Trochain 1396 (P); near Tambacounda, Herb. I.F.A.N. 8608 (IFAN).
GAMBIA: Albreda, Leprieur 5 June 1827 (P, holotype; isotypes: G, 4 sheets, P), anno 1833 (G); ibid., Perrottet 20 March 1829 (BM, G); Kombo, Heudelot 109 (P, 4 sheets, G. K; Photograph of one P sheet in K).
PORTUGUESE GUINEA: Bissau, Espírito Santo 1700 (LISC, K); Gabú, Espírito Santo 284 (COI); between Fulacunda and Bedanda, Orey 254 (K); between Cabuchangue and Quebu, Espírito Santo 2071 (COI, LISC).
SOUDAN: Kita, Bouiko, Dubois 158 (P, B).
GUINEA: Conakry, Martine 130 (= Chillou 2833) (IFAN); ibid., Chevalier 12152 (P, WAG), 12162 (P, 2 sheets, type of *A. frezoulsii*); ibid., cult. in garden Chevalier 13037bis (P); Kaloum, Conakry, Maclaud 39 (P); between Timbo and Conakry, near Kora R., Pobéguin 765 (P); Fouta Djallon, Pobéguin 1918 (P); between Konkauré and Timbo, Chevalier 12453 (P); Kouroussa, Pobéguin 841 (P).
SIERRA LEONE: Musaia, Deighton 5481 (K, MO); ibid., Miszewski 6 (K); Njala, Deighton 5960 (K, with fl. in spirit coll.); sin. loc., Deighton 1809 (K).
IVORY COAST: Forêt de l'Agnéby, W. of Dabou, Herb. I.D.E.R.T. 3140 (ABI), 3672 (ABI); ibid., Leeuwenberg 3178 (WAG).
NIGERIA: Lagos, Imp. Inst. 10 (K).

11. **A. microphylla** Wernham in Cat. Talbot's Nigerian Pl. 67. 1913; Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 18. 1931.
Fig. 14; Map 11

Type: Nigeria: Calabar, Oban, Talbot 304 (BM, holotype).
Heterotypic synonyms: *A. micrantha* Gilg et Mildbr. ex Hutch. et Dalz., l.c. (in clavi); Gilg & Mildbr. in Wiss. Ergebn. Deutsch. Zentr. Afr.-Exped. 1910–'11. 2: 189. 1922 (nomen). Type: Fernando Po: Mildbraed 6434 (holotype not seen, destroyed in B; isotype: one corolla in BM!).

A. macrocalyx Philipson in Exell, Cat. Vasc. Pl. S. Tomé 245, f. 17. B. 1944, p.p. excl. Chevalier 13690 and 13691; Monod, Bull. I.F.A.N. Sér. A. 22: pl. 3. 1960. Type: São Tomé: between Vanhulst and S. Nicolau, Exell 304 (BM, holotype; isotypes: BR, COI).

A. stenantha Philipson, l.c. p. 247, f. 17. A. Type: São Tomé: above Infante D. Henrique, Exell 626 (BM, holotype; isotype: COI).

Liana, shrub, or tree (*teste* Talbot), without spines. Twigs sometimes slightly angular when dry. Leaves shortly petiolate; blade dark green above, paler beneath, when dry greenish-brown, oblong-elliptic or oblong-obovate, 2–3 × as long as wide, 7–20 × 3–9 cm; veins inconspicuous. Sepals rounded or truncate, when dry spreading or not, the outer ones orbicular, 4–8 × 4–8 mm, the inner ones usually larger, up to 1.5 × as long as the others and up to 8 × 8 mm, often torn by the development of the corolla. Corolla in the mature bud 3–5 × as long as the calyx, 22–25 mm long, and as in the young bud tapering at the apex, white; tube 1.5–3 × as long as the calyx, 1–1.2 × as long as the lobes; lobes 9–12, oblong, spreading. Dry berry subglobose, dented or not, thin-walled.

Twigs when dry 3–9 mm thick; internodes 1–6 cm long. *Leaves:* petiole up to 0.1 × as long as the blade and up to 18 mm long; blade shortly acuminate or apiculate at the apex, rounded or cuneate at the base or decurrent into the petiole, entire. *Inflorescence* 3 × branched, 7–10 × 10–14 cm. Peduncle, branches, and pedicels not thickened. Upper bracts very small, triangular, about 1–2 × as long as the diameter of the corresponding branch, obtuse. Corolla tube cylindric or amply infundibuliform, 10–13 mm long, 3–4 mm wide at the base, 4–8 mm at the throat; lobes 2.5–3 × as long as wide, 10–12.5 × 4–5 mm, obtuse. Filaments entirely connate; anthers about 6 × as long as wide, 8–9 × 1.5 mm, sagittate at the base. Ovary obovoid- or ovoid-cylindric, about 2 × as long as wide, 6–7 × 2.5–3 mm; stigma approximately globose, 3 mm long, emarginate at the apex. Dry berry 1.2–1.5 × as long as wide, 20–30 × 15–20 mm, apiculate. Seeds dark brown, obliquely ovate-orbicular, about 1.2–2 × as long as wide, 1.5–2 × 1–1.5 mm.

Distribution: S. Nigeria, Fernando Po, Principe, São Tomé.
Ecology: Secondary forests. Alt. 400–1000 m or less (?).

NIGERIA: Calabar, Oban, Talbot 304 (BM, holotype).
FERNANDO PO: Mildbraed 6434 (BM, isotype of *A. micrantha*).
PRINCIPE: above Infante D. Henrique, Exell 626 (BM, COI, type of *A. stenantha*).
SÃO TOMÉ: S. Thalmo, Watt 7125 (BM, paratype of *A. macrocalyx*); Nova Moka, Espírito Santo 153 (COI, BM); West of Pico, Monod 11897 (BM, BR); between Vanhulst and S. Nicolau, Exell 304 (BM, BR, COI, type of *A. macrocalyx*); Porto Alegre, Chevalier 14187 (P, paratype of *A. macrocalyx*); s.n. (P, paratype of *A. macrocalyx*); southwest region, Chevalier 14492 (P, paratype of *A. macrocalyx*); sin. loc., Henriques 10 (COI, paratype of *A. macrocalyx*).

In all specimens examined the number of the corolla lobes was never less than 9. As the leaves and the sepals do not give differential characters either, the present author reduces *A. micrantha*, *A. macrocalyx*, and *A. stenantha* to synonymy. Moreover the relation of the corolla tube and lobes varies independently from the width of the corolla tube, and the size of the sepals.

12. **A. scandens** J. D. Hook., Journ. Linn. Soc. 6: 16. 1862; Baker in Fl. Trop. Afr. 4(1): 542. 1903; Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 18. 1931.

Fig. 15 (1-3)

Type: Fernando Po: Clarence Peak, Mann 623 (K, holotype).

Heterotypic synonym: *A. exelliana* Monod, Bull. I.F.A.N. Sér. A. 19: 347, f. 1–30. 1957; op. cit. 22: 54, pl. 4. 1960. Type: São Tomé: Monte Calvario, Monod 11839 (BM, 2 sheets, holo- and isotype).

Liana or tree (*teste* Exell), 6–15 m high, without spines. Twigs quadrangular when dry. Leaves petiolate; blade dark green above, hardly paler beneath, oblong-elliptic or oblong-obovate, 1.7–2.2 × as long as wide, 6–20 × 2.5–11 cm; veins inconspicuous. Sepals large, green, rounded, not spreading when dry, orbicular, 2–3 × 2–3 cm, torn by the development of the corolla. Corolla in the mature bud 2.5 × as long as the calyx, 50–75 mm long, and as in the young bud tapering at the apex, white; tube 1.5–1.75 × as long as the calyx, 1.5 × as long as the lobes; lobes 11–14 (28 in Exell 328), oblong, spreading. Dry berry subglobose, apiculate or not, thick-walled.

Twigs 4–10 mm in diam. when dry. *Leaves:* petiole short, 10–30 mm long; blade apiculate or rounded at the apex, cuneate at the base or decurrent into the petiole, entire; secondary veins 6–8 pairs. *Inflorescence* 1–3 × branched, about 1.5–2 × as wide as long, 5–10 × 9–15 cm. Peduncle, branches, and pedicels often lenticellate, short, not thickened. Upper bracts 1–3 × as long as the diameter of the corresponding branch, about 1–1.5 × as long as wide, 7–15 × 7–10 mm. Calyx often subtended by one pair of bracteoles like the upper bracts. Corolla tube 30–45 mm long, gradually widened, about 10 mm wide at the base, about 15–20 mm at the throat; lobes 2.5 × as long as wide, 20–30 × 8–12 mm, obtuse. Filaments entirely connate; anthers linear-lanceolate, 6–7 × as long as wide, 13–19 × 1.5–3 mm, sagittate at the base. Ovary ovoid-conical, about 1.5–1.7 × as long as wide, 9–10 × 5–7 mm; stigma globose, 2.5–3 mm in diam., emarginate. Dry berry hard, 4.5 × 4.1 mm, rugulose, dented or not.

Distribution: Cameroons, Fernando Po, São Tomé.

Ecology: In montane rain forests. Alt. 1200–1800 m.

CAMEROONS: Bamenda Province, Bafut-Ngemba Forest Reserve, Onochie FHI 34852 (K); ibid., Daramola FHI 40516 (K).

FERNANDO PO: Clarence Peak, Mann 623 (K, type); Moka, Exell 814 (BM).

SÃO TOMÉ: Mt. Calvario, Exell 320 (BM), 328 (BM, corolla only); ibid., Monod 11839 (BM, 2 sheets, type of *A. exelliana*); W. of Pico, Monod 11997bis (BM); Pico, Mann 1067 (K, P); Vanhulst, Macambrará, Exell 416 (BM, COI, both corollas only); Lagõa Amelia, Exell 223 (BM); Porto Alegre, Chevalier 13690 (P, veg., paratype of *A. macrocalyx*), 13691 (P, veg., paratype of *A. macrocalyx*).

In all characters *A. exelliana* falls within the individual variation of *A. scandens* and cannot be maintained as a distinct species.

13. **A. obanensis** Wernham in Cat. Talbot's Nigerian Pl. 67. 1913; Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 18. 1931.

Fig. 16; Map 12

Type: Nigeria: Calabar, Oban 305 (BM, holotype; isotypes: K, Z).

Liana, climbing shrub, or tree (*testibus* Talbot 305 et Gutzwiller 2584), unarmed. Leaves shortly petiolate; blade dark green and glossy above, paler beneath, oblong-obovate or oblong-lanceolate,

2–4.5 × as long as wide, 4.5–19 × 1–7 cm; veins inconspicuous. Sepals oblong, 1.5–2 × as long as wide, 12–17 × 6–11 mm, conspicuously concave, rounded, torn and often loosing the apex by the development of the corolla, appressed to the corolla and later to the fruit when dry. Corolla in the young bud pale yellow and tapering at the apex, in the mature bud about 2–2.3 × as long as the calyx, 24–30 mm long, and tapering, white (?); tube 1.3–1.8 × as long as the calyx, 2 × as long as the lobes; lobes 10, oblong, spreading. Dry berry ellipsoid, rugulose or not, rather thick-walled.

Twigs 3–7 mm in diam. when dry. Internodes 1.5–8 cm long. *Leaves:* petiole up to 12 mm long; blade acuminate or apiculate at the apex, cuneate at the base or decurrent into the petiole, entire. *Inflorescence* 2-3 × branched, 4–10 cm long. Peduncle, branches, and pedicels short, thick, usually lenticellate. Upper bracts oblong-triangular, 2–4 × as long as the corresponding branch, obtuse. Calyx subtended by a pair of bracteoles of about the same size and shape as the bracts. Corolla tube conspicuously widened above the calyx, 16–20 mm long, 5–6 mm wide at the base, 7–10 mm at the throat; lobes 2 × as long as wide, 8–10 × 4–5 mm, rounded at the apex. Filaments entirely connate; anthers about 5 × as long as wide, about 6 × 1.2 mm, sagittate at the base. Ovary ovoid-cylindric, about 5 × 2.5 mm; stigma subglobose, 2 × 2 mm, emarginate at the apex. Dry berry about 1.3 × as long as wide, 22–25 × 16–18 mm, apiculate. Seeds medium brown, obliquely ovate-orbicular, 1.2–2 × as long as wide, 1.5–2 × 1–1.2 mm.

Distribution: S. Nigeria, Cameroun, Congo.

Ecology: In moist places, swamps or on river banks, in rain forests. Alt. up to 1650 m.

NIGERIA: Benin: Sapoba, Jamieson R., Keay FHI 28079 (K, with fl. buds in spirit coll.); ibid., Onochie FHI 34272 (K); Calabar: Oban, Talbot 305 (BM, K, Z, type); from Oron to Eket, Talbot 3025 (BM, K); Eket District, Talbot 3105 (BM).

CAMEROUN: Bipindi, Zenker 1952 (HBG, K).

CONGO: EQUATEUR: Monkoto, Tshuapa District, Dubois 118 (BR).
KIVU: Walikale Territory: Kishanga, Léonard 2496 (BR); Muyilya, Mutongo, Gutzwiller 2584 (BR); near Bitso, Luindi R., Pierlot 1915 (BR).

14. **A. laxiflora** Bak., Kew Bull. 1895: 99; in Fl. Trop. Afr. 4(1): 542. 1903. **Fig. 15** (**4-6**)

Type: Rio Muni: near Kougué R., Mann 1802 (K, holotype; isotypes: K, P).

Climbing shrub, 4.50 m high, without spines. Leaf-blade dark (?) green above, paler beneath, when dry greenish-brown, dull. Sepals connate at the base, oblong-triangular, 15 × 10 mm, acute, keeled. Flower bud covered by the outer sepals which are closed like a beak. Corolla and stamens unknown. Dry berry globose, 2 cm long, apiculate, smooth.

Twigs lenticellate, about 5 mm in diam. when dry. Internodes conspicuous. *Leaves* decussate, shortly petiolate; petiole 8–12 mm long; blade oblong, 2.5–3 × as long as wide, 12–20 × 5–7 cm, shortly acuminate, rounded or cuneate at the base, entire. *Inflorescence* 3 × branched, 15–20 cm long. Peduncle, branches, and pedicels lenticellate, not or scarcely thickened at the nodes. Upper bracts triangular, about 1–2 × as long as the diameter of the corresponding branch, acute. Seeds obliquely ovate-orbicular.

Distribution: Once collected in Rio Muni.

RIO MUNI: near Kougué R., Mann 1802 (K, holotype; isotypes: K, P).

In *Anthocleista* hybrids may occur. A few specimens are intermediate between two species, but as they are usually incomplete their identity remains often doubtful.

Plants which differ only from the typical *A. schweinfurthii* by having spines are considered as to belong to this species.

One specimen, Louis 15602 from Congo bearing flower buds, is intermediate between *A. schweinfurthii* and *A. vogelii* by the following characters:

One thick spine above each leaf axil. Leaves sessile; blade narrowed to the auriculate base; margin not recurved. Sepals smooth, appressed to the corolla. Buds rounded or slightly tapering.

Kennedy 2131 from South Nigeria may be a hybrid of *A. schweinfurthii* and *A. djalonensis*. It has only flower buds which are approximately like those of *A. djalonensis*. In the other characters it does not differ from *A. schweinfurthii.*

Deighton 3202 from Sierra Leone consists of a vegetative branch with spines like those of *A. vogelii* but in its leaves resembles *A. procera.* Both *A. procera* and *A. vogelii* are swamp-inhabiting species. Deighton 3202 was collected in a similar locality.

Kersting A 627 from Togo has the branches and leaves of *A. djalonensis* but the calyces of *A. liebrechtsiana.*

CONGO: ORIENTALE: Yangambi, Louis 15602 (BR), *A. schweinfurthii* × *A. vogelii*?

S. NIGERIA: Benin, near Jamieson R., Sapoba, Kennedy 2131 (A, BM, MO), *A. schweinfurthii* × *A. djalonensis* ?

SIERRA LEONE: Baoma, Deighton 3202 (K), *A. vogelii* × *A. procera* ?

TOGO: Ikuwondem (?), Kersting A 627 (Z), *A. djalonensis* × *A. liebrechtsiana* ?

Doubtful species

A. inermis Engl. in Engl. Bot. Jahrb. 8: 63. 1887; Baker in Fl. Trop. Afr. 4(1): 541. 1903, p.p. quoad typum; Bruce, Kew Bull. 56. 1955.

Type: Angola: Zaire District, Island near Ponta de Lenha, Naumann 6 Sept. 1874 (holotype not seen, destroyed in B; no isotype seen).

This species could be identical with *A. liebrechtsiana* by the size of the sepals and the habitat. In the other characters its description agrees also with *A. schweinfurthii.* As there is no conclusive evidence, the present author follows Miss BRUCE in treating it as a *nomen dubium.*

Excluded species

A. brieyi De Wild., Miss. de Briey 210. 1920 = *Brenania* sp., 3 corollas preserved in spirit are *A. schweinfurthii*; herbarium specimens are *Brenania* (see Petit, Bull. Jard. Bot. Brux. 1961).

Nomina nuda

A. gabonensis hort. ex Gentil, Pl. Cult. Serres Jard. Bot. Brux. 18. 1907.

A. nigrescens Afzel. ex Gilg in Engl. Bot. Jahrb. 17: 575. 1893 = *A. nobilis* G. Don.

A. procera Leprieur ex A. Chev. var. *parviflora* A. Chev., Expl. Bot. 441. 1920, non ex Bureau = *A. nobilis* G. Don.

A. procera Leprieur ex A. Chev. var. *umbellata* A. Chev., l.c., non ex Bureau = *A. nobilis* G. Don.

Fig. 1. *A. madagascariensis:* 1. branch, ½ ×; 2–3. young flower buds, 1 ×; 4. mature bud, 1 ×; 5. flower, 1 ×; 6. portion of corolla inside, 1 ×; 7. anther, 4 ×; 8. calyx with pistil, 1 ×; 9. fruit, ½ ×; 10. seed above, 4 ×; 11. seed beneath, 4 ×; 12. longitudinal section of seed, 4 ×; 13. embryo, 12 ×. 1 (Humbert 12196 and Viguier & Humbert 1115); 2–7 (Humbert 23018): 8 (Viguier & Humbert 1115); 9–13 (Serv. Eaux For. 7609).

Fig. 2. *A. urbaniana:* 1. branch, ½ ×; 2–3. leaves, ¼ ×; 4–6. flower buds; 7. flower; 8. fruit. 4–8, ½ ×. 1–2, 4–7 (Lam & Meeuse 5805); 3 (Service des Eaux et Forêts 393); 8 (Serv. Eaux For. 2369).

Fig. 3. *A. amplexicaulis:* 1. inflorescence; 2–3. young flower buds; 4. expanding bud; 5. flower; 6. fruit; 7. portion of inflorescence. 1–7, ½ ×. 1 (Baron 3795); 2 (Decary 7393); 3 (Serv. Eaux For. 686); 4–5 (Serv. Eaux For. 7388); 6 (Lam & Meeuse 5666); 7 (Perrier de la Bâthie 8600).

Fig. 4. *A. nobilis:* 1. inflorescence (de Wilde 496); 2. branch (Leeuwenberg 3168); 3–4. young flower buds; 5. mature bud; 6. flower; 7. calyx with pistil (very young fruit); 8. fruit. 3–8 (Leeuwenberg 2322). 1–8, ½×.

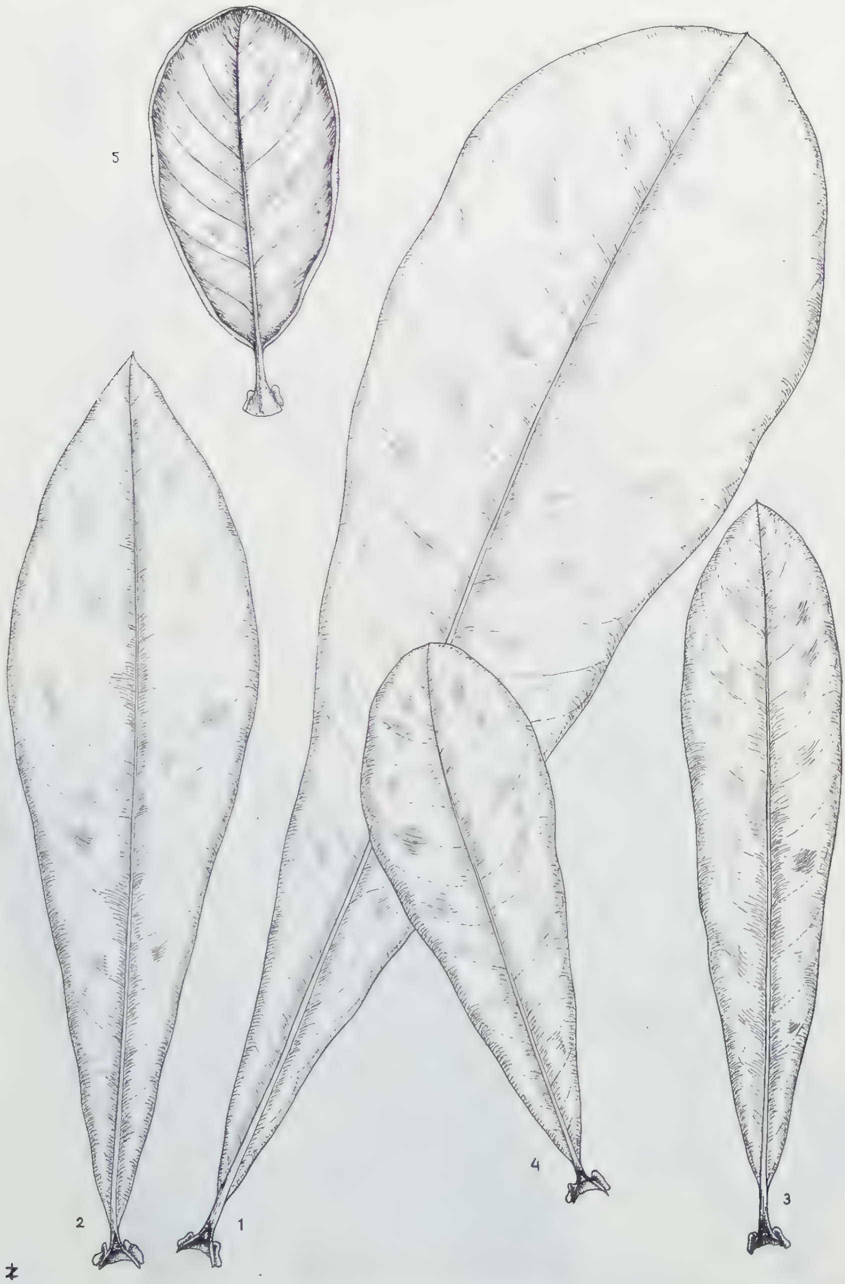


Fig. 5. *A. nobilis:* leaves, ¼ ×, 1–3. above (Leeuwenberg 1921), 4. beneath (Leeuwenberg 3168). See also Photographs 2, 3, and 5.

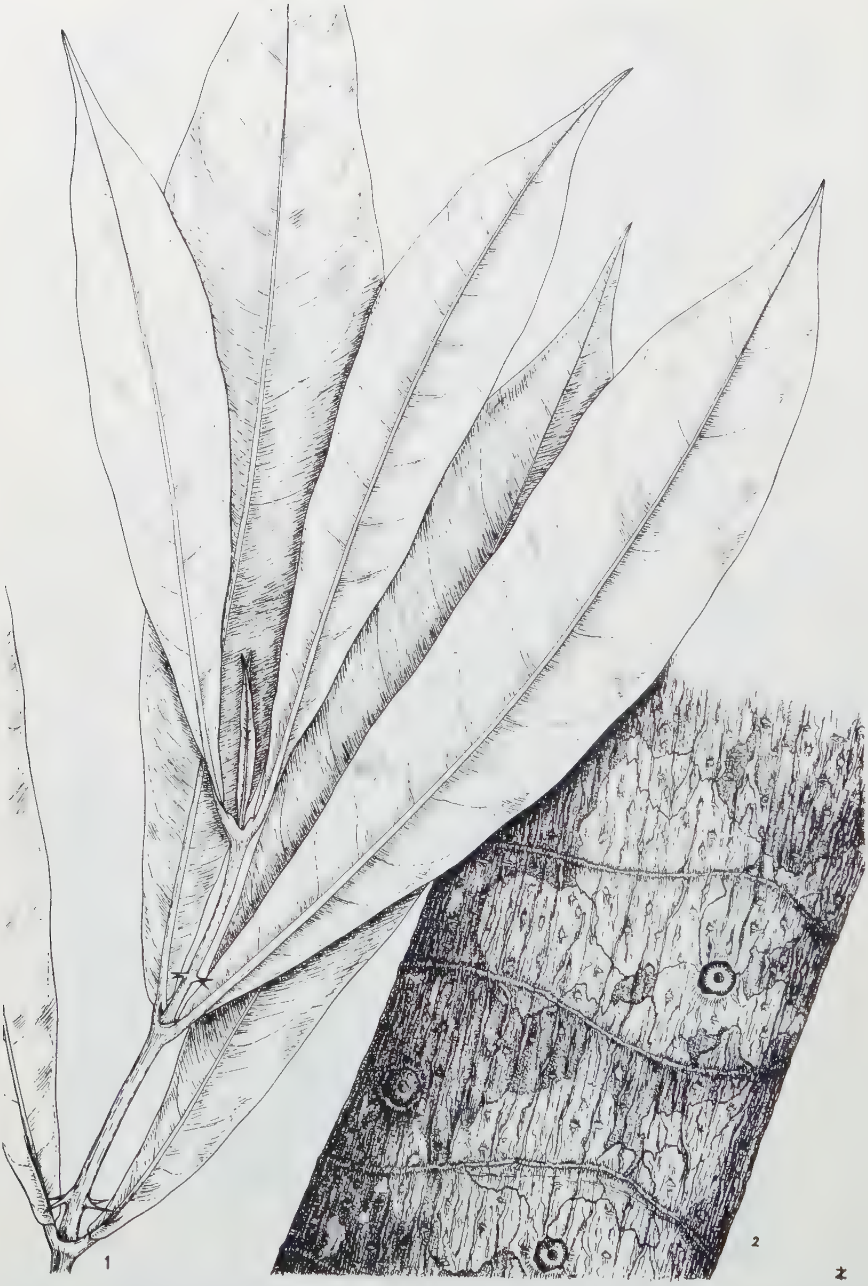


Fig. 6. *A. nobilis:* 1. apex of young plant, ½ × (Leeuwenberg 3084); 2. portion of trunk, ½ × (Leeuwenberg 2322).

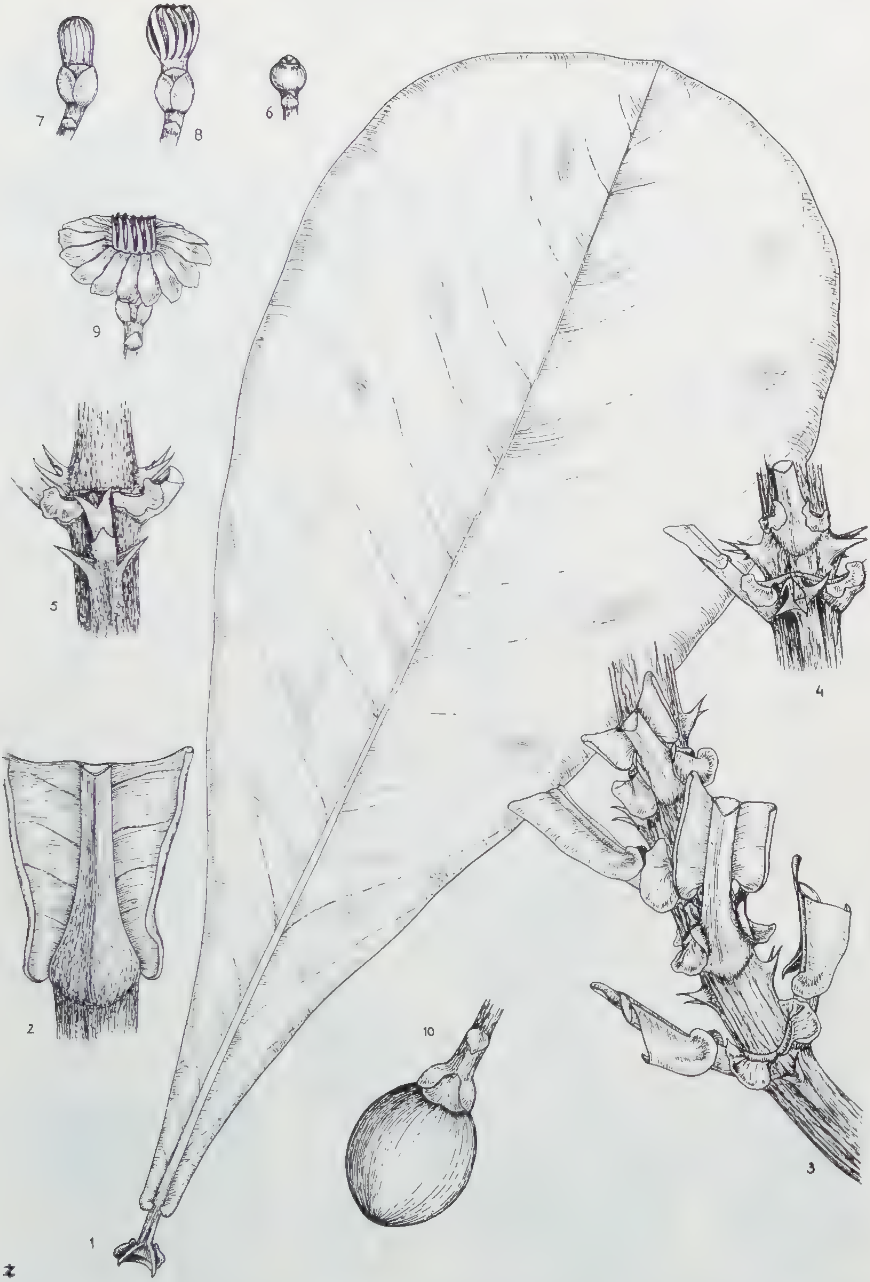


Fig. 7. *A. vogelii:* 1. petiolate leaf; 2. base of sessile leaf beneath; 3. branch with leaf bases; 4–5. branch with developing axillary buds; 6. young flower bud; 7. nearly mature bud; 8. expanding bud; 9. flower; 10. fruit. 1–10, ½ ×. 1, 4–5 (Zenker 1965); 2, 6–10 (Ghesquière 4943); 3 (Louis 13544).

Fig. 8. *A. djalonensis:* 1. branch; 2. portion of branch; 3–4. young flower buds; 5. mature bud; 6. flower; 7. portion of corolla inside; 8. calyx with pistil; 9. pistil; 10. fruit; 11. transverse section of fruit. 1–6, 8, 10–11, ½ ×; 7, 1 ×; 9, 2 ×. 1–2 (Leeuwenberg 3315); 3–7, 10 (Leeuwenberg 3316); 8–9, 11 (Leeuwenberg 3285). See also Photographs 4 and 5.

Fig. 9. *A. djalonensis:* 1–5. leaves, ¼ ×; 6–8. anthers, 3 sides, 2 ×; 9. longitudinal section of ovary, 4 ×; 10. transverse section of ovary, 4 ×; 11. ovule, 40 ×; 12. seeds, 4 ×; 13. longitudinal section of seed, 4 ×; 14. embryo, 12 ×; 15. sepal inside, with colleters, 4 ×; 16. bracts with colleters inside, 4 ×; 17. colleters, 20 ×. 1, 4 (Leeuwenberg 3167); 2–3, 5 (Leeuwenberg 3315); 6–8, 10, 15–17 (Leeuwenberg 3316); 9, 11–14 (Leeuwenberg 3285).

Fig. 10. *A. djalonensis* (seedling of Leeuwenberg 3285, cult. greenhouse, Wageningen): 1. seedling, ½ ×; 2–5, apex, 2 ×, 5. above.

Fig. 11. *A. liebrechtsiana:* 1. branch; 2–3. young flower buds; 4. expanding bud; 5. flower; 6. fruit. 1–6, ½ ×. 1–3 (Lebrun 1672); 4–5 (Leemans 219); 6 (J. Léonard 675).

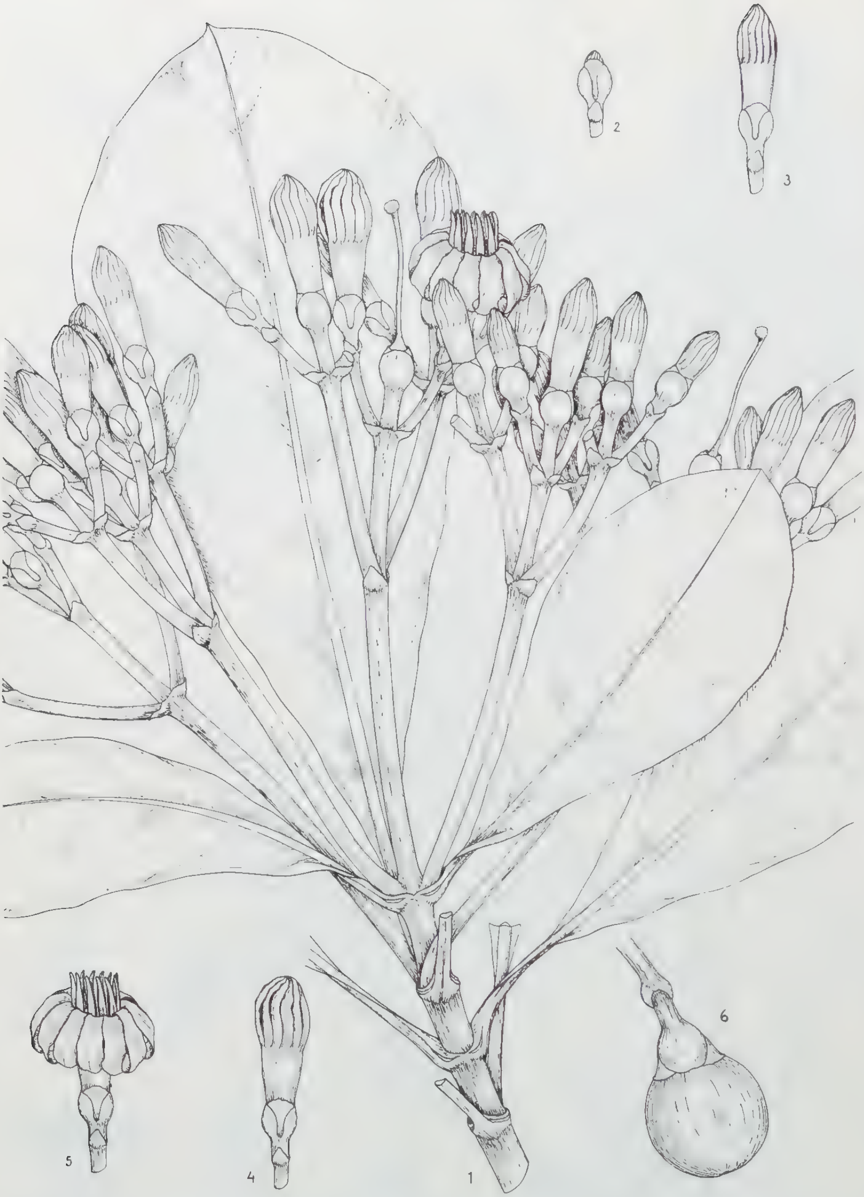


Fig. 12. *A. schweinfurthii:* 1. branch; 2–3. young flower buds; 4. mature bud; 5. flower; 6. fruit. 1–6, ½ ×. 1–3, 5 (Schmitz 3332); 4 (Tisserant 1934); 6 (Louis 3100).

Fig. 13. 1–7. *A. procera:* 1. leaf, ¼ ×; 2–3. young flower buds; 4. mature bud; 5. flower; 6. calyx with pistil; 7. fruit. 1 (Leeuwenberg 3178); 2 (Leprieur 5 June 1827); 3–6 (Chev. 12152); 7 (Dubois 158). See also Photographs 1 and 6. 8–13. *A. grandiflora:* 8–9. young buds; 10. mature bud; 11. flower; 12. calyx with pistil; 13. fruit. 8–9 (Humblot 311); 10–11 (Mendonça 287); 12 (Buchanan 84); 13 (Lebrun 4421). 2–13, ½ ×.

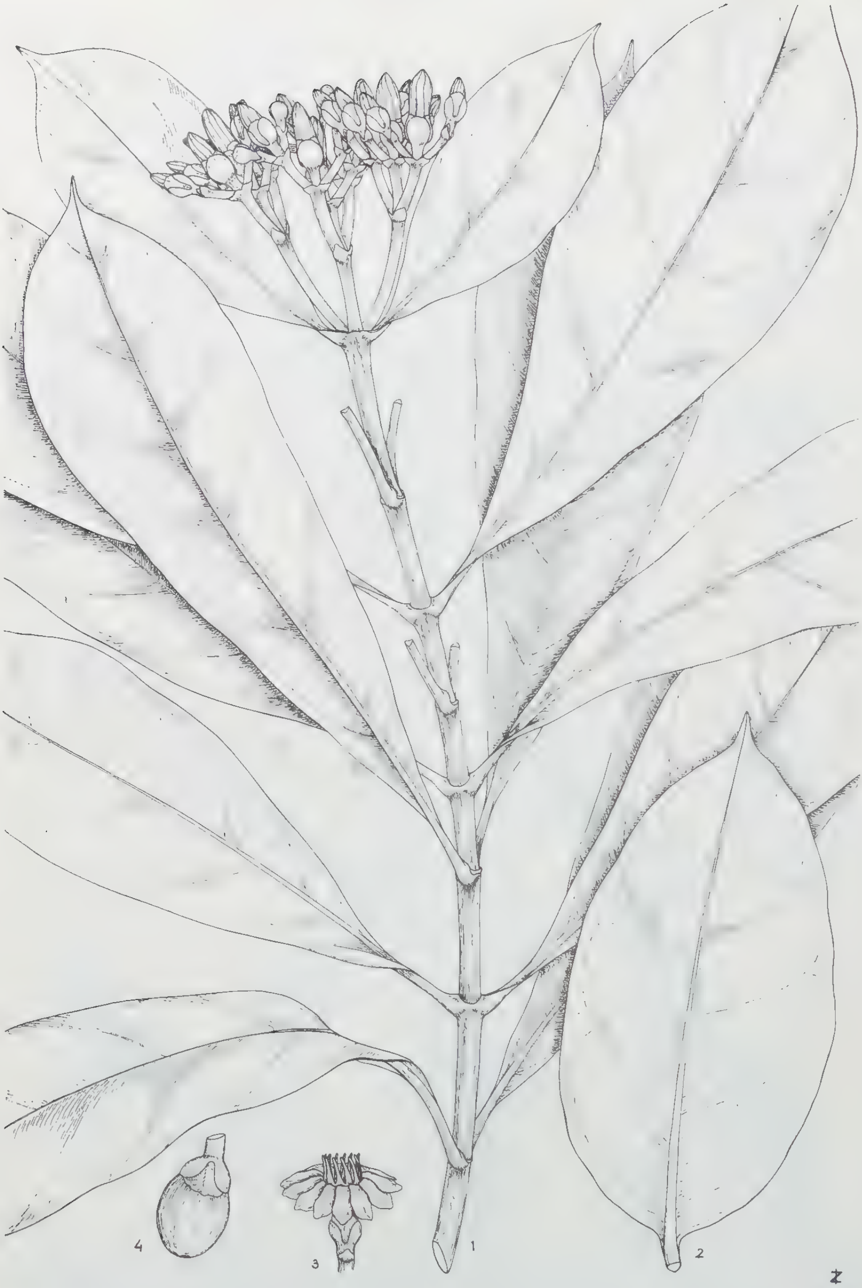


Fig. 14. *A. microphylla:* 1. branch; 2. leaf; 3. flower; 4 fruit. 1–4, ½ ×. 1 (Exell 626); 2–3 (Monod 11897); 4 (Espírito Santo 153).

Fig. 15. 1–3. *A. scandens:* 1. branch; 2. leaf; 3. fruit. 1 (Mann 623); 2 (Onochie FHI 34852); 3 (Monod 11997bis). 4–5. *A. laxiflora:* 1. leaf; 2. fruit. 1–2 (Mann 1802). 1–5, ½ ×.

Fig. 16. *A. obanensis:* 1. branch; 2. inflorescence; 3–5. flower buds; 6. flower; 7. calyx with pistil; 8–9. immature fruits; 10. fruit; 11–12. leaves. 1–12, ½ ×. 1 (Onochie FHI 34272 and Talbot 305); 2–4 (Keay FHI 28049); 5–6 (Talbot 305); 7, 9 (Dubois 118); 8, 12 (Talbot 3025); 10 (Pierlot 1915); 11 (A. Léonard 2469).

INDEX OF NAMES

(Synonyms are in *italics*. Page numbers of principal entries in **bold face**; those of figures in *italics*.)

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NOTES ON MYXOMYCETES III

A NEW SPECIES FROM SURINAME

N. E. NANNENGA-BREMEKAMP

(*Doorwerth*)

(*received September 6th, 1960*)

Physarum mennegae nov. spec. maxime ut *Ph. penetrale* Martin, sed sporangiis subglobosis, non distincte elongatis, stipite luteolo, non rubro, concretionibus calcareis albis, non luteis, capillitio minus denso et non persistente, sporis majoribus ab eo recedens; typus: 945 in collectione auctoris, lectus a Dr. A. M. W. Mennega in Guiana Batavorum Centrali.

Sporangia gregaria, stipitata, e hypothallo orbiculari parvo orientia, altitudine 1 mm non excedentia. Hypothallus decolor, translucens. Stipes sporangio fere aequilongus vel eo paulo longior, fragilis, pallide luteolus, translucens, lucem orientem versus visus luteus, interdum paulo in sporangii cavitatem productus. Sporangium subglobosum, circ. 0.5 mm diam., brunneum; peridium sine concretionibus calcareis, tenue, translucens, cum lucem orientem versus visum est decolor, irregulariter dehiscens; capillitium laxius, e filamentis gracilibus, hyalinis, in reticulum connectis compositum; filamenta nodis calcareis albis, nunc globosis, nunc ramificatis instructa. Sporae subglobosae, 7–8 μ diam., per saturam saturate brunneae, lucem orientem versus visae pallide violaceo-brunneae, minutissime verruculosae, verruculis per greges conjunctis. Plasmodium ignotum.

Habitat partem centralem Guianae Batavorum, ubi in foliis mortuis crescit.

Sporangia gregarious, stipitate, rising from a small circular hypothallus; height not exceeding 1 mm. Hypothallus colourless and transparent. Stipe about equal in length to the sporangium or slightly longer, brittle, pale yellowish, translucent and yellow by transmitted light, occasionally penetrating a little way into the sporangium. Sporangium subglobose, about 0.5 mm in diam., brown; peridium without lime concretions, thin, transparent, colourless by transmitted light; dehiscence irregular; capillitium not very dense, consisting of fine, hyaline, anastomosing threads with white, rounded or branched lime knots, not persistent. Spores in mass dark brown, by transmitted light pale violaceous brown, subglobose, 7–8 μ in diam., very minutely warted; the warts grouped in clusters. Plasmodium unknown.

Central Suriname: Upper part of the Tanjimama Creek (a tributary of the Coppename River), Dr. A. M. W. Mennega, Nov. 1955. "on dead leaves".

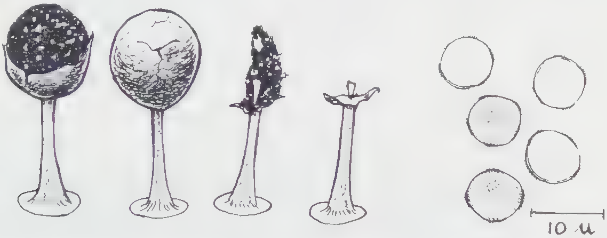

Fig. 1. *Physarum mennegae.* Sporangia and spores; three of the latter in optical section.

The type consists of over a hundred sporangia, some of which are mouldy. Part of the material was sent by me to Dr. Martin with the request to give me his opinion on the position of this specimen with regard to *Ph. penetrale*, which seemed to me to come closest to it. Dr. Martin kindly studied it, and wrote to me that he very much doubted its identity with this species, because in the latter the sporangia are noticeably taller than wide, instead of subglobose; to this difference I can add that the stipe is yellow and not red as in *Ph. penetrale*, that the capillitium is less dense and not persistent, that the lime knots are white instead of yellow, and that the spores are larger.

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NOTES ON MYXOMYCETES IV
MYXOMYCETES COLLECTED IN THE NETHERLANDS, CHIEFLY IN THE VICINITY OF DOORWERTH (GELDERLAND)

N. E. NANNENGA-BREMEKAMP

(*Doorwerth*)

(*received September 6th, 1960*)

This is the first of a series of short papers dealing with Myxomycetes collected by me since August 1951 in the Netherlands, mostly in an area not exceeding 6 square kilometers in extent, situated round Doorwerth in the province of Gelderland, and extending from Heelsum in the West to Oosterbeek in the East, and from Wolfheze in the North to the banks of the Rhine in the South. Localities will only be mentioned by name when the specimens were collected outside this area, or when they appear to be rare here.

Species which according to Prof. Dr. W. K. H. Karstens (private communication) have not been found previously in the Netherlands, have been marked with an asterisk. The (unpublished) list of Dr. Karstens, which covers the whole of the Netherlands, contains 18 species that were not found by me.

Specimens of all species included in my list are preserved either in my private collection or in that of the Botanical Museum and Herbarium of the State University, Utrecht (in the last-mentioned case the number is followed by a "U") or in both.

I would like to thank the Keeper of Botany of the British Museum of Natural History, London, for allowing me to study the Lister collection, and the staff of the Cryptogamic Department of the British Museum for their kind help. I am also indebted to Dr. D. A. Reid and Dr. R. G. Dennis of the Kew Herbarium for their assistence during my visit to that Institute, and especially to Prof. Dr. G. W. Martin for his help and critical remarks, and for the exchange of named specimens, and to Prof. Dr. W. K. H. Karstens for his advice and for the permission to use the above mentioned list, which comprises all Myxomycetes that were known to him from the Netherlands up to 1953. After that date very little has been added by other authors to our knowledge of the Netherlands Myxomycetes.

Ceratiomyxa Schröt.

Ceratiomyxa fruticulosa (Müll.) Macbride.

Very common on decaying wood (11, 107; 172; 238; 249; 283 U; 610; 683; 954 U; 976; 986; 1166; 1183; 1283; 1233 v. infra; 1861 U; 1884 U; 1894 U; 1896; 1909; 1910; 1921, "var. *flexuosa*" v. infra; 1957 U; 1976; 2000 U; 2043 U; 2116 U; 2131; 2219; 2570; 2575, "var. *flexuosa*" v. infra; 2583; 2622/3, "var. *flexuosa*" v. infra; 2628, 2629 U; 2639 U; 2681: 2801 U; 2816 U; 2862 U; 2870 U; 2872; 2993; 3033; 3069; 3731; 3981 U; 4171 U; 4243).

Very variable in shape: almost simple to intricately branched, the branches sometimes reticulately anastomosing; in the sporophores: from flat and rather broad to slender, white, pale ochraceous or

salmon-coloured, with a more or less prominent areolation, and practically without granules or with a rather large number of granules in scattered groups; in the spore stalks: terete (round in optical section) or flattened (oval in optical section); and in the spores: usually prolate, but sometimes subglobose, smooth or with minutely granulate contents (in 1233 coarsely granulate, v. infra).

Quite a number of varieties have been described in this species. The numbers 1921, 2219, 2575, 2622, 2623 and 2801 answer the description of var. *flexuosa* Lister, but it seems to me that this variety can not be kept up as intermediate forms occur.

It is perhaps of interest to note that the spore stalks or spicules, the connection between the spore and the sporophore, differ in length. In the monographs published by the Listers, by Macbride and Martin and by Hagelstein no attention is paid to their length, but in the "North American Flora" Martin calls them "slender" and "uniform", in contrast to those of *C. sphaerosperma* Boedijn, where those at the tip of the sporophore branches are said to be several times as long as the lateral ones. In all specimens of *C. fruticulosa* collected by me part of the spore stalks are two to four times as long as the others (Fig. 1 a, b and c), and these longer ones are scattered over the whole sporophore.

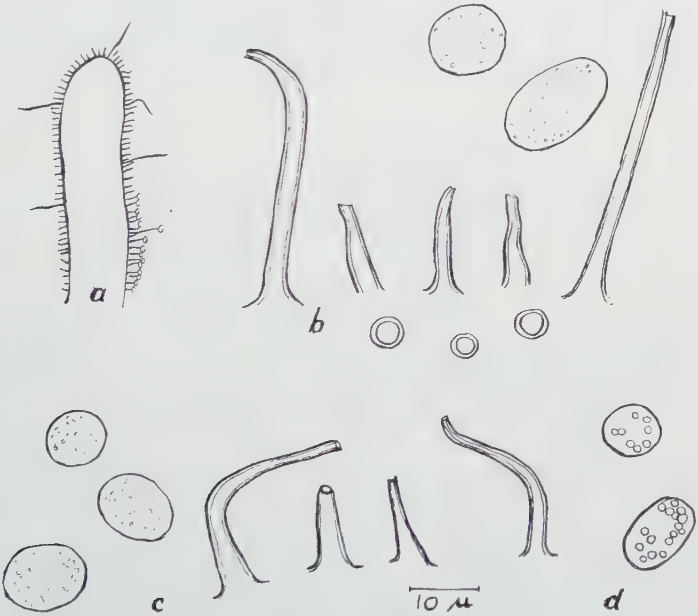

Fig. 1. *Ceratiomyxa fruticulosa* (Müll.) Macbride: a. sketch of a sporophore in longitudinal section, showing length and distribution of spore stalks; b. spore stalks (three seen from above) and spores (4368) in entirely fresh (moist) condition; c. the same after a few hours of gentle drying; d. spores of 1233.

In 1233 the contents of the spores are not smooth or very minutely granulate, but coarsely granulate or warted (Fig. 1 d). Part of this

specimen was sent to Dr. Martin, who kindly studied it for me, and suggested that the difference might be due to the spores not being perfectly mature. In view of the large number of specimens in which no spores of this kind were found, this did not look very probable to me, but as the possibility could not be excluded, it seemed advisable to study the spores of sporangia in different stages of development. To this end large plasmodia of *Ceratiomyxa* were collected, and placed in a moist chamber. At different stages parts were taken out and left to dry. The result was that in none of the different samples (i.e. in 3981 with white sporophores, and in 4243 with salmon-coloured ones) spores were found that resembled those of 1233; they were all of the ordinary kind, i.e. with finely granulate contents. Even spores that were taken from a still moist and transparent sporophore proved to be indistinguishable from those found in a fully mature part. It seems probable, therefore, that 1233 is different, but as the difference seems to be confined to this point, it does not seem worth varietal distinction.

Tubifera J. F. Gmel.

Tubifera ferruginosa (Batsch) J. F. Gmel.

Mostly on dead stumps of coniferous trees (18; 66 U; 147; 175 U, collected at Bilthoven; 191 U; 321 U; 430; 565 U; 742; 1015; 1150; 1250; 1311; 1569; 1981; 2012 U; 2021 U; 2114; 2393; 2755; 2790; 2878, collected at Zeist; 2998; 3003; 3496); more rarely on dead wood of other trees (93 U, on *Salix*; 2021, on *Betula*; 3153, on *Prunus*).

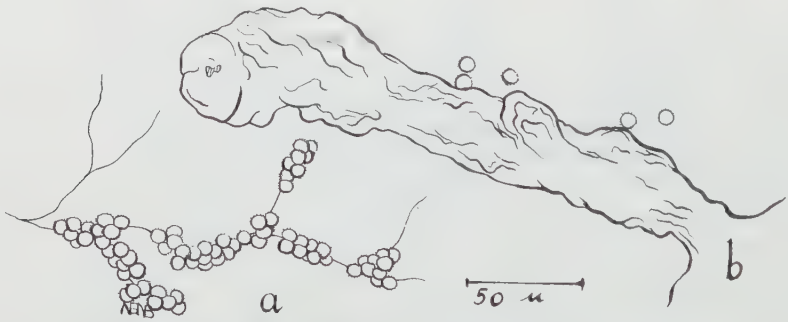

Fig. 2. *Tubifera ferruginosa* (Batsch) J. F. Gmel.: a. pseudo-capillitium with adhering spores (93); b. tubule projecting inward from peridium (4135).

The identification of these specimens offered some difficulties. In all of them part of the sporangia composing the pseudo-aethalia contain either slender threads of a pseudo-capillitium (Fig. 2 a), rigid tubuli suspended from the peridium (Fig. 2 b), or columellae; sometimes two of these structures are present in the same pseudo-aethalium, and occasionally even all three. The structure of the pseudo-aethalium itself varies too. In a large number of specimens the sporangia are entirely free in the upper half, and closely appressed in the lower one. On account of the presence of the pseudo-capillitium and columellae

identification with the keys given in the monographs of the Listers, of Macbride and Martin and of Martin would lead to their inclusion in *T. casparyi* (Rost.) Macbride, as in that species pseudo-capillitium is present, and as in about a third of the sporangia composing the pseudo-aethalium columellae are found, whereas *T. ferruginosa* is said to be "destitute of any trace of columella and capillitium". However, in a specimen probably collected in Iowa and identified by Macbride, of which Dr. Martin kindly sent me a part, a few slender threads of pseudo-capillitium are present, so that the absence of the latter can not be regarded as a general character.

Another difference between the two species is said to be found in the size of the spores. According to Macbride those of *T. casparyi* measure 7.5-9 μ in diam., whereas those of *T. ferruginosa* are 6–8 μ in diam.; those of *T. casparyi*, moreover, are said to be darker and more thoroughly reticulate. This difference in the surface of the spores is not mentioned by Martin in the "North American Flora", where the spores of both species are described as "reticulate over three-fourth of the surface". C. J. Alexopoulos (in Brittonia **10**: 27. 1959) describes the spores of two Greek specimens of *T. ferruginosa* as "more uniformly reticulate than is usual for the species", while K. S. Thind and M. S. Manocha (in Indian Phytopathology **11**: 19–20. 1958) describe a specimen from the Mussoorie Hills as having the spores 5.2–6.4 μ in diam.; as no mention is made of a capillitium, the latter was presumably absent, as according to the literature, it should be.

In my specimens the spores look exactly like those figured by Lister for *T. casparyi*, i.e. they are practically all of them reticulate for about seven eighths of their surface, the remaining part being brokenly reticulate, or reticulate with wider meshes; in size they agree, however, with those of *T. ferruginosa*, for they are 6.5–8 μ in diam.

In the Lister collection I could study two specimens identified as *T. casparyi* (B.M. 2805, from Sweden, and B.M. 2806, from Washington). The spores proved to be about 8 μ in diam., and the peridium was papillate in the first-mentioned specimen, and nearly smooth in the second. The specimen from Washington, moreover, had the columellae branching at the tip. All in all the difference between these specimens and *T. ferruginosa* is not very marked. However, I did not see the specimen shown in Fig. 150 of Lister's monograph, which looks rather distinct, for it shows regular horizontal connections between peridium and columella, and this should probably be regarded as an important character. If the specimen figured by Lister, which was collected in the United States, really belongs to the species described by Rostafinski, it must be regarded as excluded that the specimens collected in the Netherlands could belong to the latter.

According to Macbride and Martin (Monograph) other differences are found in the peridium and in the hypothallus. The former would be minutely granular in *T. casparyi*, and thin and transparent in

T. ferruginosa; Lister, however, mentions in *T. ferruginosa* the presence of warts or papillae on the inner surface of the peridium, but gives no information on the structure of the peridium in *T. casparyi*; in my specimens smooth, warted or papillose peridia are present. The hypothallus is described as strongly developed, white and spongiose in *T. ferruginosa*, and as thin, brown and explanate in *T. casparyi*; in my own specimens they are spongiose, never extending beyond the aethalium. The structure of the hypothallus may prove to be the best diagnostic feature to separate the two species.

Another difference between the two species lies in the colour of the plasmodium. That of *T. ferruginosa* is described by all the above-mentioned authors as passing through a beautiful rose colour, whereas that of *T. casparyi* apparently does not show this bright hue, but is described as white changing into a dull grey, and finally becoming umber.

In a quite considerable part of the specimens collected by me, the development of the pseudo-aethalia out of the plasmodium could be studied, and in all of them the latter appeared to pass through a stage in which it showed a bright orange or salmon colour. For this reason too it seemed to me that they ought to be referred to *T. ferruginosa*. This conclusion seems to be confirmed by the fact that the peridium is either smooth or papillate, but never granular, as that of *T. casparyi* is said to be. It must be admitted, however, that this character is perhaps not fully reliable, as in the two specimens of the Lister collection that were studied by me, the peridium was not granular either.

The spores in my material measure 6.5–8 μ in diam., and this too is in agreement with the descriptions given of *T. ferruginosa*, but as there is some overlapping in the size of the spores, this character can not be regarded as decisive.

The greatest difficulty is caused by the fact that some of my specimens have columellae and a liberal amount of pseudo-capillitium, in fact 93 looks quite woolly inside, but it seems to me that the variability in respect to this character may be greater than hitherto has been assumed. Therefore, unless we place ourselves on the standpoint that a larger number of species has to be distinguished in this group, and for this standpoint there seems to be no sufficient ground as yet, it seems best to include our material in *T. ferruginosa*.

Macbride and Martin mention the occurrence of a membrane covering the pseudo-aethalia of *T. casparyi*. Among my own collections 1311, 2755, and part of one of the four aethalia of 2012 show a broken film, not unlike the one which covers the aethalium of *Reticularia*. This, and the presence of the slender *Reticularia*-like pseudo-capillitium, seem to point to an affinity of *Tubifera* with the Reticulariaceae, rather than with the Liceaceae, where this genus is placed by Martin in the "North American Flora". *Tubifera* is doubtless related to *Alwisia*, which was included by Martin in the Reticulariaceae. It has a similar pseudo-capillitium as *Tubifera*, but its sporangia, though connected at the base in a common stalk, are free.

However, in some specimens of *Tubifera* the sporangia are also nearly free.

In my opinion the delimitation of the family Reticulariaceae ought to be widened so as to include the genera *Alwisia*, *Tubifera*, *Dictyaethalium*, *Enteridium* and *Lycogala*. It would then be characterized by sporangia combined into aethalia or pseudo-aethalia and, at least, provided with a common stalk, by the structure of the but rarely lacking pseudo-capillitium, and in that of the outer covering.

The strong resemblance between *Alwisia* and *Tubifera* has already been pointed out, that between *Tubifera* and *Dictyaethalium* is also very striking, the main point of difference being that in *Dictyaethalium* the walls between the sporangia have disappeared for the most part. *Reticularia* resembles *Tubifera* in the structure of the pseudo-capillitium and in some cases in that of the cortex, and *Enteridium* shows a very near affinity with *Reticularia*. The tubules which in *Lycogala* replace the threadlike pseudo-capillitium found in the other genera, occur side by side with the latter in *Tubifera*.

Craterium Trent.

The genus *Craterium* is represented in my collection by five species from the Netherlands, four from the vicinity of Doorwerth, and one from Bilthoven (prov. of Utrecht). Two species and one variety are new records for the Netherlands, and one (*Cr. concinnum*) was apparently not yet recorded from Europe.

Craterium minutum (Leers) Fries.

On dead leaves and straw (193 U; 198 U; 329 U; 369 U; 377; 383 U; 398; 634; 711 U; 740 U, 978; 1295; 1302; 1369; 1395, Bilthoven; 1750; 1825; 1855; 1862; 2268; 2274; 2317; 2353; 2960; 3085; 3150; 3184; 3288; 3294; 3304 U).

The cup as well as the stalk always show a bright yellow to orange tint by transmitted light. Occasionally in some of the sporangia belonging to the same colony the cups are confluent (377, 3288, 3298), and with one exception (part of 1855) the lid is well defined; in the aberrant part of 1855 the lid is absent, and here the sporangia dehisce irregularly at the top, but in other sporangia of this colony the lid is normally developed. The spores show but little variation, and this is observed in the other species of this genus too. The plasmodium is bright vermillion or orange.

* *Craterium concinnum* Rex.

On dry leaves at Bilthoven (1927).

This seems to be the first record of this species for Europe. The small, brown sporangia were found in a dry ditch under bracken and oak in the "Eykensteinse bossen" near Bilthoven. The collection consists of a dozen sporangia.

The peridium is thin, brown and glossy, and lacks the bright orange tints that are always present in my specimens of *Cr. minutum*. The spores are somewhat darker and more distinctly violaceous than those of *Cr. minutum*.

Craterium leucocephalum (Pers.) Ditmar var. *leucocephalum*.

On dead leaves and stems of herbs (373; 640 U; 713; 1385 U, Bilthoven; 1462; 1588; 1613; 1636; 4211; 4291; 4295 U; 4405 Ewijk).

The collections are all typical of the species, and as usual in this genus, the spores show but little variability. The plasmodium, where seen, was yellow.

* *Craterium leucocephalum* (Pers.) Ditmar var. *scyphoides* Lister.

Collected on dead leaves in a wood adjacent to the "Ridder Robertlaan", Doorwerth (1604; 4175 U; 4227).

Of most of the sporangia of 1604 only the red-brown cups with tattered edges, the spores and the nearly white lime knots are left; a few sporangia, however, are intact, and show the nearly white, farinose upper half. The other specimens are better, and show the diverse stages; in some the pseudo-columella projects in the centre, while the peridium has crumbled away to a lower level. The spores of this variety are larger than those of the typical form, i.e. about 10–11 μ, instead of 7–9 μ, a difference not mentioned in any of the monographs quoted in this paper.

* *Craterium cylindricum* Massee.

On a milk bottle cap between dead leaves (2108, leg. J. L. Nannenga).

Lister considered this species to be a variety of *Cr. leucocephalum* (Pers.) Ditmar. Macbride and Martin (the Myxomycetes, 1931) returned it to its former position, but Martin (North American Flora, 1948) considered it conspecific with *Cr. leucocephalum*. As it differs from the latter in the shorter stalk, the smooth peridium, and the paler, more yellowish spores, it seems to me that it deserves to be recognized as a distinct species.

***Craterium auronucleatum** nov. spec. maxime ut *Cr. leucocephalum* ad quod praesertim corpusculis crystallinis luteis peridio et capillitii nodis innatis accedit, sed sporangiis minoribis, turbinatis vel subglobosis, capillitio denso e filamentis gracillimis composito, filamentis nodis calcareis valde ramosis instructis, pseudocolumella clare aurantiaca, sporis colore saturatiore tinctis ab eo distinguenda; typus 4491, lectus prope villem "Kabeljouw" in vico Wolfheze (Fig. 3).

Sporangia gregaria, sessilia vel stipite ad 0.2 mm longo instructa, stipite incluso 0.4–0.7 mm alta, 0.3–0.5 mm diam., quoque sporangio e hypothallo orbiculari oriente. Hypothallus translucens, ad lucem orientem versus visus clare aurantiacus. Stipes, si adest, plicatus, aurantiacus vel brunneus, lucem orientem versus visus semper aurantiacus, sine inclusionibus granulatis. Sporangium (Fig. 3, a and b) subglobosum vel turbinatum, in parte inferiore aurantiacum, in parte superiore nunc saturate et ferventer brunneum, nunc praesentia inclusionum calcalrearum remissius et dilute aurantiacum. Peridium tenue, translucens, fere semper corpuscula crystallina lutea continens et inde nunc totum aurantiacum, nunc in parte inferiore solum

aurantiacum et in parte superiore ecoloratum; inclusionibus calcareis, si adsunt, in parte superiore solum repertis; dehiscentia (Fig. 3, c) circumscissilis, ut in *Craterio leucocephalo* cupulam margine plus minusve inaequali instructam relinquens. Capillitium (Fig. 3, d) e filamentis

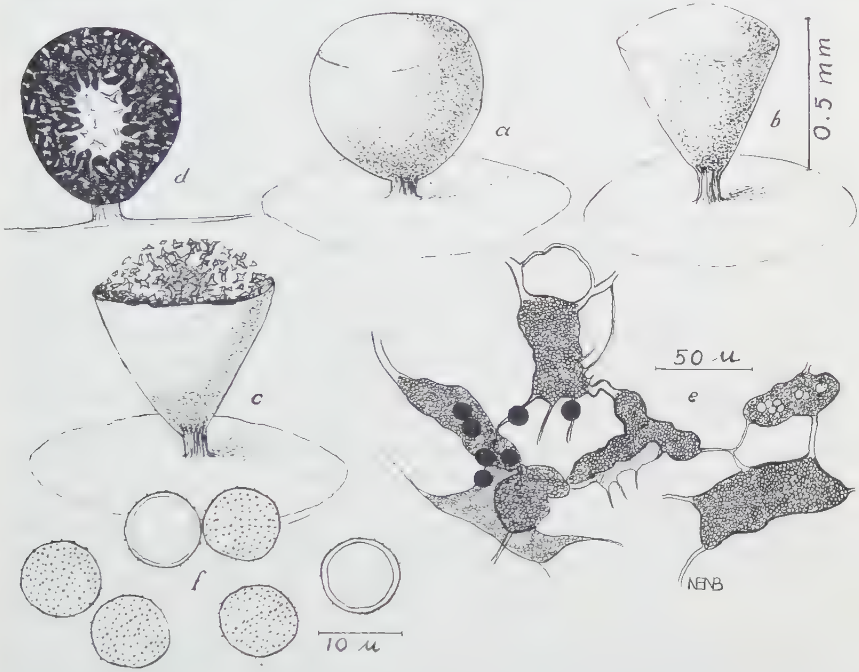

Fig. 3. *Craterium auronucleatum* nov. spec.: a. and b. sporangia; c. dehisced sporangium; d. sporangium in longitudinal section; e. capillitium nodes and spores; the node on the right showing the yellow corpuscles; f. spores.

gracillimis permultis albis, hic inde nodis parvis albis vel interdum luteolis vel dilute aurantiacis, margine irregulariter in excrescentias graciles productis instructis compositum (Fig. 3, e); nodi si quantitatem calcis parvam continent, margine calce carent, casu quo excrescentiis plerumque luteolis compressis; corpuscula lutea quae peridio intersunt, in nodis etiam occurrunt; in sporangii centro nodi in pseudocolumellam clare aurantiacam, superficie irregularem confluunt. Sporae (Fig. 3, f) globosae, 9–10 μ diam., per saturam saturate brunneae, ad lucem orientem versus visae saturate violaceo-brunneae, minute verruculosae. Plasmodium ignotum.

Habitat rivi vallem prope villam "Kabeljouw" in vico Wolfheze, ubi in area circ. 1 m in quadrato metiente et e foliis mortuis et e herba viva consistente plures coloniae parvae repertae sunt (N. E. Nannenga–Bremekamp 4491 U).

Sporangia gregarious, sessile or with an up to 0.2 mm long stipe, with the stipe 0.4–0.7 mm high and 0.3–0.5 mm in diam.; each

sporangium rising from an orbicular hypothallus. Hypothallus transparent, clear orange by transmitted light. Stipe, when present, plicate, orange or brown, translucent orange by transmitted light, without granular inclusions. Sporangium (Fig. 3, a and b) subglobose or turbinate, in the lower part orange, in the upper part either dark glossy brown or, on account of a sprinkling of lime, dull light orange. Peridium thin, transparent, almost always with yellow crystalline corpuscles, either orange throughout or in the lower part only, the upper part being colourless; inclusions of lime, when present, confined to the upper part; dehiscence (Fig. 3, c) circumscissile, leaving, as in *Craterium leucocephalum*, a cup with a more or less uneven edge. Capillitium (Fig. 3, d) dense, consisting of a large number of slender white filaments with small white, or sometimes yellowish or light orange, calcareous nodes (Fig. 3, e); when the specimens contain but little lime, the latter is confined to the peseudo-columella, and then the nodes are expanded and flat and show, as a rule, a pale yellowish tint; the yellow crystalline corpuscles that are found in the peridium, occur also in the nodes on the filaments; in the centre of the sporangium the nodes are fused into a bright orange pseudo-columella with unequal surface. Spores (Fig. 3, f) globose, 9–10 μ in diam., dark brown in mass, dark violaceous brown by transmitted light, minutely warted. Plasmodium unknown.

The type (N. E. Nannenga-Bremekamp 4491 U) was collected in the valley of the brook at Wolfheze near the farm "Kabeljouw" on dead leaves and living grass under a solitary oak. It was part of a group of many small colonies scattered over an area of about 1 square meter (8.9, 1960).

This new species comes close to *Craterium leucocephalum*, which it resembles among other things in the presence of yellow crystalline corpuscles in the peridium and in the capillitium nodes. It differs from that species in the smaller size and the turbinate or subglobose shape of the sporangia, the dense capillitium consisting of very thin threads with many branched lime knots, the brilliant orange pseudo-columella and the darker colour of the spores.

The yellow corpuscles found in the periderm and in the nodes on the capillitium threads were regarded by Lister as a characteristic feature of *Cr. leucocephalum* and the two varieties of this species distinguished in the monograph, viz. *cylindricum* and *scyphoides*.

Craterium aureum (Schum.) Rost.

In summer on dead leaves and straw, and on living leaves of ivy (372; 382 U; 1301 b; 1308; 1368; 1394 U, Bilthoven; 1438; 1455; 2838 U; 2839; 2890, Bilthoven; 2899; 2904; 2907; 2959; 3012); on fallen bark of apple (4247, sporangia somewhat abnormally developed; spores normal).

The sporangia are usually turbinate, and differ therefore from those of * *Physarum flavidum* (Peck) Peck, with which I have found it growing in mixed clumps. The spores of the latter are, of course, much darker and larger. Those of *Cr. aureum* are rather uniform. The plasmodium, seen in a number of cases, was yellow.

Echinostelium De Bary

* *Echinostelium minutum* De Bary

Very common on the bark and wood of dead and living trees. The sporangia are in their natural habitat difficult to see, and were therefore all obtained from moist-chamber cultures from the bark of living trees of *Quercus*; 3326; 3327 U; 3329; 3330; 3349; 3412; 2417; 3568, Plasmolen near Nymegen; 3866 U; 3881; 3882; 3885; 3877; 4092; of *Betula*: 3712; 3728; 3804; of *Prunus laurocerasus*: 4131; 4312; of *Aesculus*: 3863; 3856; of *Fagus*: 3306; of *Larix*: 3715; from a fallen branch of *Tilia*: 3664 U; 3669; 3671; 3700; from decaying coniferous wood: 3332; 3806; 3903; 3946/3953; 4051).

When the stalk has been developed, the "plasmodium" shows as a hyaline drop. Most of my specimens are white or nearly so; 3728, 3885 and 4131 are very pale pink. The spores all measure 6–9 μ, except those of 3306, which are about 12 μ in diameter; those of 3728 and 4092 are very minutely spinulose; those of the other specimens are smooth. They are often provided with slightly thickened "areolae", as described in Lister's "Monograph" and by Alexopoulos (Am. Journn. Bot. **47**: 38, Fig. 5). The latter says that this structure of the spore wall is a character confined to the genus *Echinostelium.*

***Echinostelium fragile** nov. spec., maxime ut *E. minutum* De Bary et *E. elachiston* C. J. Alexopoulos, a priori magnitudine triente, ab altero stipite apicem versus contracta, columella conica brunnea et sporis majoribus recedens; typus 3868, cultus e plasmodio lecto in loco dicto "Hemelse Berg" prope Oosterbeek–Laag, ubi in cortice Aesculi crevit (Fig. 4).

Sporangia sparsa vel gregaria, stipitata, erecta vel nutantia, 0.13–0.15 mm alta. Stipes apicem versus sensim contractus (Fig. 4 a), decolor, interdum striatulum, basi substantiam granulosam paucam continens, circ. 90 μ longus. Sporangium globosum, 30–50 μ diam., dilute rosea vel salmonea, post dies paucos colorem griseo-brunneum sumens; peridium vix distinguendum, evanescens, sed ad apicem stipitis annulum distinctum relinquens; columella (Fig. 4 b) brunnea, fusiformis, circ. 6 μ longa; capillitium nullum; sporae (Fig. 4 c) subglobosae, per saturam necnon lucem orientem versus visae dilute

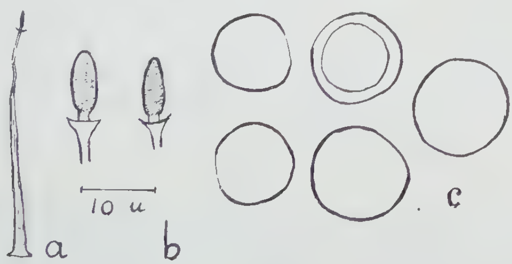

Fig. 4. *Echinostelium fragile* nov. spec.: a. stipe with peridial collar and columella; b. two columellae enlarged; c. spores (enlarged to the same scale as b); the two spores on the left and the three spores on the right from two different sporangia (both belonging to the type colony); one of the spores in optical section.

roseae vel griseae, laeves, areola tenuiore 12–(13)–15 μ diam. instructae. Plasmodium hyalinum, dilute roseum.

Habitat provinciam Gelriam ubi in loco dicto "Hemelse Berg" prope vicum Oosterbeek–Laag in cortice *Aesculi* lectum est.

Sporangia scattered or gregarious, stipitate, erect or nodding, 0.13–0.15 mm high. Stipe (Fig. 4 a) tapering, colourless, sometimes slightly striate, with a little included matter at the base, about 90 μ long. Sporangium globose, 30–50 μ in diameter, very pale pink or salmon, turning brownish-grey after a few days; peridium almost invisible, evanescent, leaving a tiny, well-marked collar at the tip of the stipe; columella (Fig. 4 b) brown, fusiform, about 6 μ long; capillitium absent; spores (Fig. 4 c) subglobose, pale pink or grey in mass as well as by transmitted light, smooth, with a small thinner area 12–(13)–15 μ in diam. Plasmodium hyaline, pale pink.

Netherlands: Province of Gelderland, "Hemelse Berg" near Oosterbeek–Laag (3862; 3867; 3868, type; 3915; 3916; plasmodia on the bark of *Aesculus*; sporangia grown in moist-chamber culture).

This minute species resembles *E. minutum* De Bary in general aspect, especially in the structure and in the shape of the stipe, but it is only about one third as high. Among the specimens quoted above, some sporangia of *E. minutum* itself were also present. It comes apparently also close to *E. elachiston* Alexopoulos, but differs from that species in the tapering stalk, the presence of a brown fusiform columella and the larger size of the spores. The thickenings of the spore wall are absent or so very inconspicuous as to appear so, but this was also observed in some gatherings of *E. minutum*. The small sporangia are very difficult to mount, as they become easily detached, probable owing to the absence of a capillitium. This applies also to the sporangia of *E. elachiston*.

Acta Botanica Neerlandica **10** (1961) 67–79

FLORISTISCHE NOTITIES 68–79

S. J. VAN OOSTSTROOM EN TH. J. REICHGELT
(*Rijksherbarium, Leiden*)

(*ingekomen 15 november 1960*)

In deze serie Floristische Notities [1]) worden in hoofdzaak de aanwinsten van de Nederlandse flora uit het jaar 1959 behandeld. Zij werden ook reeds in het kort vermeld in de lijst van voor Nederland nieuwe vondsten in De Levende Natuur **63**, 1960, p. 161–164.

De meeste van de hier opgenomen soorten, nl. de nrs. 69, 70, 72, 73, 74, 76, 77 en 78 zijn te beschouwen als door de Maas aangevoerd. Zij zijn vrijwel zeker afkomstig van de wolfabrieken aan de Vesdre in België, vanwaar vruchten of zaden met het water van Vesdre en Maas werden meegevoerd, evenals dat met vroegere vondsten aan de Maasoevers in Limburg het geval was. Zie hiervoor de inleiding van onze serie Floristische Notities 35–58 (2) en de verdere hieronder vermelde literatuur (1, 3, 4, 5).

De nummers 77 en 78 werden bewerkt door de heer J. H. Kern, Rijksherbarium, Leiden.

1. S. J. VAN OOSTSTROOM & TH. J. REICHGELT, Adventieven aan de Maasoevers in Limburg. Corr. bl. Rijksherb. **1**, 1956, p. 8–10.
2. ———, Floristische Notities 35–58. Acta Bot. Neerl. **7**, 1958, p. 33–52.
3. ———, Adventieven langs de Maas in Limburg. Natuurhist. Maandbl. **47**, 1958, p. 67–70.
4. ———, Adventieven langs de Maas in Limburg (Een kleine aanvulling). Natuurhist. Maandbl. **48**, 1959, p. 89.
5. ———, Adventieven langs de Maas in Limburg, II. Natuurhist. Maandbl. **49**, 1960, p. 19–22.

68. **Alnus glutinosa** (L.) Vill. f. **quercifolia** (Willd.) Koehne

Aan het kanaal Almelo-Nordhorn, ca. 1–1½ km ten O. van „De Pook", gem. Tubbergen, leg. *Chr. Smeenk*, 18 okt. 1959 (herb. L).

Van *Alnus glutinosa* zijn verschillende laciniate vormen in cultuur. Bovenvermeld exemplaar, waarvan de bladen vrij diep en onregelmatig gelobd zijn, komt het meest overeen met f. *quercifolia* (Willd.) Koehne. BOOM (1, p. 115) vermeldt, dat deze vorm in ons land niet zelden wordt gekweekt. Volgens de vinder is echter het exemplaar van Tubbergen daar ter plaatse niet aangeplant, doch oorspronkelijk.

1. B.K. BOOM, Flora der Cultuurgewassen van Nederland **1**, ed. 4, Wageningen, 1959.

69. **Dysphania myriocephala** Benth. (Fig. 1)

Itteren, L., in een verlaten grintgroeve aan de Maas, ten N. van het dorp, leg. *J. H. Kern, S. J. van Ooststroom* & *Th. J. Reichgelt*, 2 sept. 1959, herb. *v.O.* no. 21726 (herb. L).

[1]) Eerdere series werden gepubliceerd in Acta Bot. Neerl. **5**, 1956, p. 102–114; **5**, 1956, p. 322–334; **7**, 1958, p. 33–52 en **9**, 1960, p. 197–207.

Deze uit Australië afkomstige soort is reeds een paar maal in Europa (Duitsland, België) als woladventief aangetroffen. Zij is als volgt gekarakteriseerd:

Plant kruidachtig, op stengel en bladen met zeer korte, verspreide haren. Stengel aan de voet vertakt, met liggende tot opstijgende, tot ca. 40 cm lange, kantige takken. Bladen lijnvormig tot langwerpig lancetvormig, met stompe tot afgeronde top en wigvormig in de korte bladsteel versmalde voet, tot ongeveer 1(–2) cm lang, in twee rijen geplaatst. Bloemen in talrijke, dichte, zittende, okselstandige kluwens, merendeels vrouwelijk, de eindelingse van een kluwen meestal tweeslachtig; kluwens ca. 2 mm in diam. Bloemdek (Fig. 1, a en b) meestal 2-, zelden 1- of 3-(?)bladig, ca. ½ mm lang; de bladen (Fig. 1, c) bovenaan sterk kapvormig opgeblazen en over de vrucht heengrijpend, aan de voet in een nagel versmald,

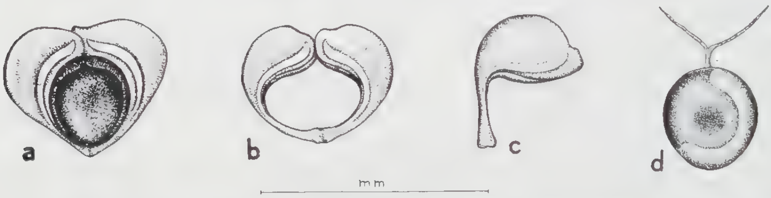

Fig. 1. *Dysphania myriocephala* Benth.; a: bloemdek met vrucht, b: idem, zonder vrucht, c: bloemdekblad, d: vrucht.

onderaan zeer kort vergroeid en bij rijpheid vaak afzonderlijk afvallend. Meeldraden 1(–2). Stijl 1, diep 2-delig, samen met de beide draadvormige stempels tot even lang als de vrucht. Vrucht (Fig. 1, d) een weinig afgeplat, in omtrek breed elliptisch tot bijna cirkelrond, ca. ⅓ mm lang. Zaad verticaal, van dezelfde vorm als de vrucht, bruin.

Het geslacht *Dysphania* R.Br. werd oorspronkelijk beschreven als nauw verwant met *Chenopodium*; later werd het door Bentham & Hooker (3) tot de *Illecebraceae* gerekend, terwijl Baillon (2), die de *Illecebraceae* bij de *Caryophyllaceae* onderbrengt, het in de laatste familie plaatst. Diverse meer recente auteurs beschouwen het weer als een *Chenopodiaceae*-geslacht; Aellen (1) gaat zelfs zover, dat hij er een sectie van *Chenopodium* van maakt. Tenslotte baseren Pax (4, p. 230) en Pax & Hoffmann (5) er de familie *Dysphaniaceae* op, met als enige geslacht *Dysphania*. Deze opvatting wordt, naar Dr. Aellen (Basel) ons schriftelijk mededeelde, ook gehuldigd in de thans verschijnende nieuwe druk van Hegi's Flora.

1. P. Aellen, Die systematische Stellung und Gliederung der R. Brownschen Gattung Dysphania. Bot. Jahrb. **63**, 1930, p. 483–490.
2. H. Baillon, Histoire des Plantes **9**, 1888, p. 129.
3. G. Bentham & J. D. Hooker, Genera Plantarum **3**, 1880, p. 14.
4. F. Pax, Zur Phylogenie der Caryophyllaceae. Bot. Jahrb. **61**, 1927, p. 223–241.
5. F. Pax & K. Hoffmann, Dysphaniaceae, in Engler & Prantl, Die natürlichen Pflanzenfamilien **16**c, 1934, p. 272–274.

70. **Amaranthus macrocarpus** Benth. var. **melanocarpus** Thell. (Fig. 2,c)

Itteren, L., aan de Maasoever ten N. van het dorp, leg. *J. H. Kern, S. J. van Ooststroom* & *Th. J. Reichgelt*, 2 sept. 1959, herb. *v. O.* no. 21762 (herb. L).

Amaranthus macrocarpus, die in verschillende Europese landen als woladventief werd aangetroffen, is afkomstig uit Australië. De soort is als volgt te karakteriseren:

Plant eenhuizig, kaal. Stengels liggend, tot ca. 25 cm lang. Bladschijf eirond tot ruitvormig, met duidelijk uitgerande top, ca. ½–1 ½ cm lang, aan de voet geleidelijk in de ongeveer even lange steel versmald. Bloemkluwens alle in de bladoksels, dicht, in de vruchttijd halfbolvormig tot bijna bolvormig, met naar alle zijden uitstaande bloemen. Bloemen met 3 bloemdekbladen en met in vorm weinig daarvan verschillende, gewoonlijk iets kortere schutbladen. Bloemdekbladen der vrouwelijke bloemen smal spatelvormig met kort topspitsje tot lijn-priemvormig, spits, breed tot smal vliezig gerand. Vrucht (Fig. 2, c) ongeveer spoelvormig, ca. 2 ½–4 mm lang, ca. 1 ½–3 maal zo lang als het bloemdek, sterk gerimpeld tot bijna glad, zwart, rood- of witachtig, niet openspringend. Zaad omgekeerd eivormig, met stompe rand, ca. 1 mm lang.

Ons exemplaar behoort tot de var. *melanocarpus* Thell. met de volgende kenmerken:

Bloemdekbladen smal spatelvormig, breed vliezig gerand, aan de top vrij stomp, met topspitsje. Vrucht met dunne wand, zwart, sterk gerimpeld.

71. **Lepidium pseudo-didymum** Thell. (Fig. 2,a)

Tilburg, terrein bij wolfabriek Bern. Pessers, leg. *A. W. Kloos Jr*, 12 juli 1950 (herb. L); idem, terrein bij Tilburgse Wolwasserij, leg. *A. W. Kloos Jr*, 11 aug. 1950 (herb. L); idem, terrein bij wolfabriek Bern. Pessers, leg. *J. H. Kern, S. J. van Ooststroom* & *Th. J. Reichgelt*, 1 sept. 1958, herb. *v. O.* no. 21020 (herb. L).

In THELLUNG's monografie van het geslacht *Lepidium* (5) wordt *L. pseudo-didymum* nog niet vermeld. Zij werd pas later door deze auteur in een artikel van DRUCE (1, p. 308) beschreven op grond van planten, die in 1913 als woladventief werden aangetroffen in Schotland bij Galashiels aan de Tweed. Zie ook HAYWARD & DRUCE (2, p. 23, fig. 6) en lit. 6.

L. pseudo-didymum behoort tot de grex *Bipinnatifida* Thell., een soortengroep, die in Amerika voorkomt, en Thellung vermoedt, dat de soort uit Zuid-Amerika afkomstig is. Behalve Galashiels, waar zij in de jaren 1913–1916 werd aangetroffen, vonden wij geen andere adventieve vondsten vermeld. In sept. 1958 troffen wij de soort aan als woladventief te Tilburg, waarbij de grote habituele gelijkenis met *Coronopus didymus* (L.) Sm. opviel. Dit bracht ons ertoe de in het Rijksherbarium onder deze laatste naam liggende exemplaren eens zorgvuldig door te zien. Hierbij kwamen de twee boven geciteerde, door Kloos in 1950 te Tilburg verzamelde, planten te voorschijn.

Een korte beschrijving van de soort volgt hier:

Plant eenjarig (ook tweejarig?). Stengels liggend of opstijgend, tot ca. 50 cm lang, vrij sterk vertakt, met afstaande of iets naar beneden gerichte, ca. $\frac{1}{3}$ mm lange haren. Onderste bladen dubbel, hogere meestal enkel veerdelig, met ongeveer lijnvormige tot smal elliptische, $\frac{3}{4}$–2$\frac{1}{2}$ mm brede slippen en ongeveer even brede rhachis, aan de voet niet geoord, verspreid behaard. Bloeiwijzen aan het eind van de stengel en de takken, in de bloeitijd kort, in de vruchttijd verlengd, tot ca. 1 dm lang. Bloemen met smal eirond-lancetvormige, smal witgerande, ca. $\frac{3}{4}$–1 mm lange, gedeeltelijk op de rug behaarde, ten dele vroeg afvallende, ten dele vrij lang blijvende kelkbladen en lijn-priemvormige, witte kroonbladen, die ongeveer $^1/_2$–$^2/_3$ maal zo lang zijn als de kelk. Meeldraden 2. Hauwtjes in omtrek bijna cirkelrond of iets meer breed dan lang, ca. 2–2$^3/_4$ mm lang en 2$^1/_4$–3 mm breed, aan de voet breed afgerond, aan de top tot ca. $^1/_5$–$^1/_6$ van de lengte van het tussenschot uitgerand, met vrij spitse, evenwijdige lobben, aan de top zeer smal gevleugeld, ter weerszijden van het replum aan beide kanten iets gewelfd en netvormig gerimpeld, kaal, bij rijpheid uiteenvallend in twee helften, waarin het zaad besloten blijft; stempel bijna zittend in de insnijding. Stelen der hauwtjes ongeveer even lang als tot weinig langer dan deze, iets afgeplat en zeer smal gevleugeld, kort behaard, tenslotte zwak naar beneden gekromd. Zaden in omtrek langwerpig omgekeerd eirond, zonder vleugelrand, bruin, met bij bevochtiging verslijmende zaadhuid. Zaadlobben incumbent.

Thellung's opmerking, dat de soort vermoedelijk uit Zuid-Amerika afkomstig zou zijn, was voor ons aanleiding om de op Zuid-Amerikaanse *Lepidium*-soorten betrekking hebbende literatuur na te zien. Hierbij bleek, dat Hitchcock (3) een overzicht publiceerde over deze Zuid-Amerikaanse soorten. Determineren wij ons materiaal met de in dit overzicht gegeven tabel, dan komen wij op *L. inclusum* Schulz. Al hebben wij van deze soort geen materiaal gezien, toch bestaat er bij ons geen twijfel dat zij identiek is met *L. pseudo-didymum* Thell. De door Schulz (4, p. 189) en Hitchcock (3, p. 126, fig. 40) gegeven beschrijving en afbeelding slaan volkomen op exemplaren in het Rijksherbarium, die door Thellung werden gekweekt uit zaden, die van de planten van Galashiels afkomstig waren.

L. inclusum Schulz, in Fedde, Rept. 33, 1933, p. 189 is dus een synoniem van *L. pseudo-didymum* Thell., in Rep. Bot. Exch. Club 3, 1913 (1914) p. 308. De soort komt volgens Schulz en Hitchcock in Patagonië en op Vuurland voor. Hiermede is dus het vermoeden van Thellung betreffende de herkomst bevestigd.

Bij onderzoek van het rijke materiaal, dat wij ter beschikking hebben, blijkt, dat de kelk bij een deel der bloemen spoedig afvalt, bij andere daarentegen vrij lang blijft. Op deze wijze is te verklaren, dat Thellung de kelkbladen „subpersistentia" noemt, terwijl Hitchcock van „promptly deciduous" spreekt.

Verder schrijft Thellung, dat de zaden bij rijpheid uit de kleppen van het hauwtje vallen, terwijl Schulz opgeeft, dat de in de kleppen

ingesloten zaden samen met deze afvallen. Dit laatste hebben ook wij geconstateerd bij ons materiaal en bij dat van Thellung.

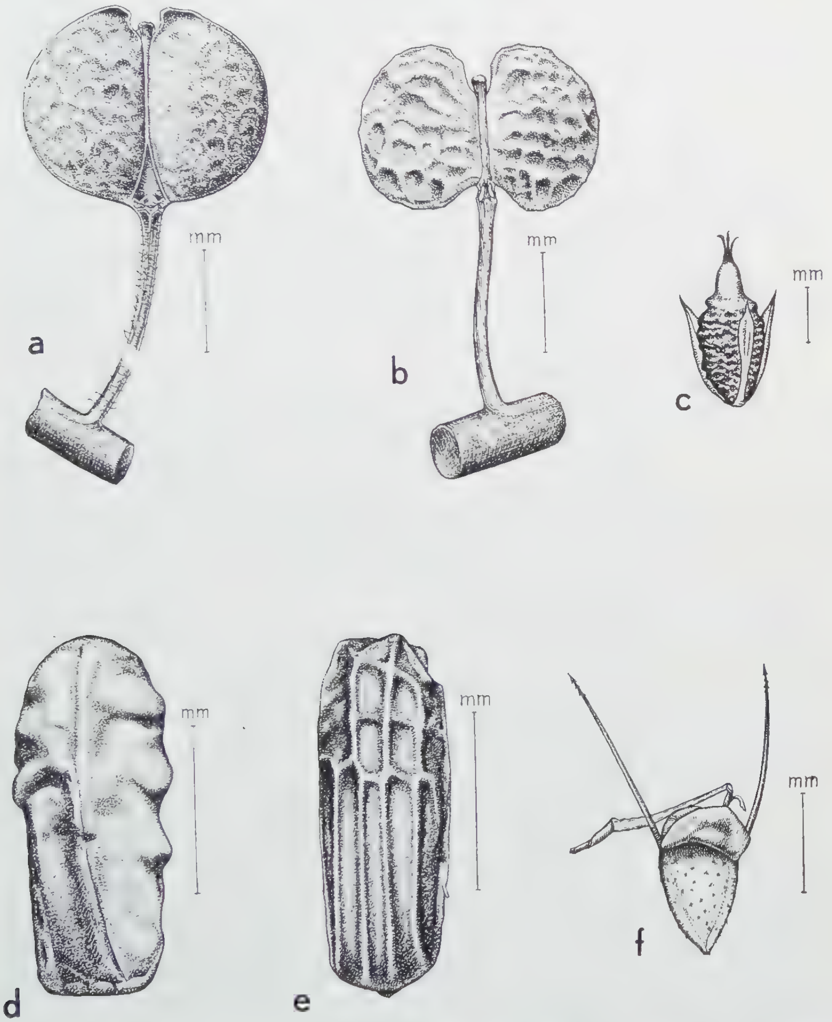

Fig. 2. a: hauwtje van *Lepidium pseudo-didymum* Thell.; b: idem van *Coronopus didymus* (L.) Sm.; c: bloemdek met vrucht van *Amaranthus macrocarpus* Benth. var. *melanocarpus* Thell. (naar Hegi); d: nootje van *Verbena supina* L.; e: idem van *V. officinalis* L.; f: vrucht van *Calotis cuneifolia* R.Br.

Tenslotte merken wij nog op, dat de in 1950 verzamelde planten veel forser zijn (tot ca. 50 cm lang) dan die welke de vroegere auteurs zagen. Deze planten hebben bovendien langere vruchtdragende trossen (tot ca. 1 dm).

Zoals wij reeds hierboven mededeelden, bestaat er in habitus een opvallende overeenkomst tussen *Lepidium pseudo-didymum* en *Coronopus*

didymus. Beide soorten zijn echter aan de hauwtjes duidelijk van elkaar te onderscheiden. Deze zijn nl. bij *Lepidium pseudo-didymum* (Fig. 2, a) aan de voet afgerond, bij *Coronopus didymus* (Fig. 2, b) hartvormig; verder zijn de kleppen bij de eerste minder sterk gerimpeld, terwijl de zaadhuid bij deze soort bij bevochtiging verslijmt, wat bij *C. didymus* niet het geval is.

1. G. C. DRUCE, Plant notes for 1913, etc. Rep. Bot. Exch. Club **3**, 1913 (1914), p. 307–376.
2. I. M. HAYWARD & G. C. DRUCE, The adventive flora of Tweedside, Arbroath 1919.
3. L. C. HITCHCOCK, The South-American species of Lepidium. Lilloa **11**, 1945, p. 75–134, 4 pl.
4. O. E. SCHULZ, Über verschiedene Cruciferen. Fedde, Rept. **33**, 1933, p. 183–191.
5. A. THELLUNG, Die Gattung Lepidium (L.) R.Br. Mitt. Bot. Mus. Univ. Zürich **28**, 1906.
6. Vermischte neue Diagnosen. Fedde, Rept. **14**, 1914, p. 78/158.

72. **Glinus lotoides** L.

Itteren, L., aan de Maasoever ten N. van het dorp, leg. *J. H. Kern, S. J. van Ooststroom* & *Th. J. Reichgelt*, 2 sept. 1959, herb. *v. O.* no. 21758 (herb. L).

Het geslacht *Glinus* L., behorende tot de *Aizoaceae*, telt ongeveer 10 soorten en komt voor in de tropische en subtropische gebieden van beide halfronden. *G. lotoides* is verspreid in de tropen en subtropen van de oude wereld en is bovendien ingeburgerd in Noord-Amerika.

De soort is als volgt gekenmerkt:

Eenjarige, dicht sterharige, kruidachtige plant. Stengel liggend of opstijgend, ca. 15–90 cm lang, gaffelvormig vertakt. Bladen tegenoverstaand of ten dele schijnbaar in kransen, omgekeerd eirond tot bijna cirkelrond, aan de top vrij spits tot afgerond, aan de voet in de steel versmald, ca. 1–3 cm lang. Bloemen in groepjes in de bladoksels, kort gesteeld. Bloemdek tot dicht bij de voet 5-delig, ca. 5–8 mm lang, met elliptische, vrij spitse, ten dele vliezig gerande, van binnen kale en geelwitte slippen. Meeldraden variërend in aantal van ca. 5 tot 15, waartussen een wisselend aantal lintvormige, vaak 2-spletige staminodiën. Vruchtbeginsel ellipsoidisch, 5-hokkig, met korte stijl en 5 stempels. Vrucht een veelzadige, met 5 kleppen openspringende doosvrucht. Zaden bruin, met regelmatige rijen wratten bezet, vrijwel zittend op de placenta en voorzien van een in omtrek ongeveer hartvormige strophiole, die in de insnijding een lang draadvormig aanhangsel draagt, dat om het zaad is geslagen.

Dit aanhangsel wordt door sommige auteurs ten onrechte als een funiculus opgevat, o.a. door PAX & HOFFMANN (2) in hun tabel op p. 190 en door FERNALD (1, p. 608).

1. M. L. FERNALD, Gray's Manual of Botany, ed. 8, 1950.
2. F. PAX & K. HOFFMANN, Aizoaceae, in Engler & Prantl, Die natürlichen Pflanzenfamilien **16**c, 1934.

73. **Verbena supina** L. (Fig. 2, d)

Itteren, L., in een verlaten grintgroeve aan de Maas ten N. van het dorp, leg. *J. H. Kern, S. J. van Ooststroom* & *Th. J. Reichgelt*, 2 sept. 1959, herb. *v. O.* no. 21738 (herb. L).

Verbena supina is naast *V. officinalis* L. de enige in Europa oorspronkelijk voorkomende soort van dit geslacht. Het areaal omvat de Kanarische eilanden, het Middellandse Zeegebied (Iberische Schiereiland, Italië, Balkan Schiereiland, noordelijk tot Hongarije; Noord-Afrika van Algerije tot Egypte) en Voor-Azië.

De soort is van *V. officinalis* te onderscheiden o.a. door de volgende kenmerken:

Stengels liggend of opstijgend, lager dan bij *V. officinalis*. Bladen dubbel veerdelig, de hogere minder gedeeld. Aren vrij dicht, in de vruchttijd veel minder verlengd. Bloemen kleiner dan bij *V. officinalis*, ca. 3–4 mm lang. Nootjes (Fig. 2, d) aan de rugzijde met een of twee onduidelijke overlangse ribben en onregelmatige grove dwarse plooien, iets langer en breder dan bij *V. officinalis*. Bij de laatste vertonen de nootjes 4 of 5 scherpe overlangse ribben, die bovenaan door scherpe dwarsribben verbonden zijn. (Fig. 2, e).

74. **Calotis cuneifolia** R.Br. (Fig. 2, f)

Itteren, L., in een verlaten grintgroeve aan de Maas ten N. van het dorp, leg. *J. H. Kern* & *S. J. van Ooststroom*, 20 juni 1959, herb. *v. O.* no. 21421 (herb. L); Tilburg, terrein bij wolfabriek Bern. Pessers, leg. *J. H. Kern*, *S. J. van Ooststroom* & *Th. J. Reichgelt*, 1 sept. 1959, herb. *v. O.* no. 21704 (herb. L).

Het geslacht *Calotis* R.Br. behoort tot de tribus *Astereae* subtribus *Asterinae* der *Compositae*. Het oorspronkelijke areaal van het geslacht is volgens DAVIS (1) geheel of vrijwel geheel beperkt tot Australië (22 soorten). Een 5-tal soorten, waaronder ook *Calotis cuneifolia*, is met Australische wol aangevoerd in verschillende landen van Europa.

De soort is als volgt gekenmerkt:

Plant overblijvend (als woladventief bij ons eenjarig), rechtopstaand of opstijgend, tot ca. 60 cm hoog, min of meer sterk behaard met gelede haren. Bladen verspreid; wortelbladen met ongeveer cirkelronde, grof gekarteld-getande schijf en slanke, aan de voet geleidelijk verbrede steel, de hogere bladen van dezelfde vorm, wigvormig in de aan de voet geoorde steel versmald, de bovenste bladen smaller, met gevleugelde en aan de voet geoorde steel. Hoofdjes eindelings en okselstandig, gesteeld, ca. 1–2 cm in diam.; omwindselbladen langwerpig, vrij spits. Lintbloemen wit of lila, ca. 3–9 mm lang en ½–1½ mm breed; buisbloemen geel. Vrucht (Fig. 2, f) afgeplat, in omtrek ongeveer omgekeerd driehoekig, ca. 1–1½ mm lang en ½–1½ mm breed, roodbruin. Pappus bestaande uit 2(3–4) in hun bovenste helft van weerhaken voorziene, ca. 1–3½ mm lange naalden, afwisselend met een gelijk aantal schubben, die meer breed dan lang zijn.

1. G. L. DAVIS, Revision of the genus Calotis R. Br. Proc. Linn. Soc. New South Wales **77**, 1952, p. 146–188.

75. **Erigeron acer** L. × **E. canadensis** L. (**E.** × **hülsenii** Vatke)

Stavoren, op een verwaarloosd spoorwegemplacement, leg. *M. T. Jansen* & *D. T. E. van der Ploeg*, 24 aug. 1959 (herb. L).

Op bovengenoemd spoorwegemplacement groeiden samen met een groot aantal planten van *E. acer* en *E. canadensis* enige tientallen exemplaren, die habitueel tussen beide soorten instonden. In bouw geleken zij meer op *E. acer* door de vrij lange, meer dan bij *E. canadensis* uitstaande takken met betrekkelijk weinig hoofdjes; deze hoofdjes waren kleiner dan die van *E. acer*, terwijl de omwindselbladen minder behaard waren dan bij deze soort, doch iets sterker en langer dan bij *E. canadensis* het geval is. De kleur der lintbloemen kwam overeen met die van *E. acer*; ze waren echter in verhouding tot de grootte van het hoofdje iets langer. Tenslotte bleken de planten niet of slecht vrucht te zetten, zodat de gevolgtrekking niet te gewaagd was, dat men hier te doen had met een bastaard van de beide *Erigeron*-soorten.

Deze hybride, die door VATKE (3) werd beschreven van de omgeving van Staykowo in het tegenwoordige Polen, wordt door HEGI (2, 1917, p. 446) vermeld van een viertal plaatsen in Duitsland, Polen en Tsjecho-Slowakije en door FLINCK (1) van Zweden.

1. K. E. FLINCK, Hybriden Erigeron acre × canadense, funnen i Skåne. Bot. Notiser 1953, p. 144.
2. G. HEGI, Illustrierte Flora von Mittel-Europa **6**(1), 1913–18.
3. VATKE, Erigeron Hülsenii Vatke. Ein neuer Bastart aus der Posener Flora. Österr. Bot. Zeitschr. **21**, 1871, p. 346.

76. **Matricaria intermedia** (Hutchinson) Van Ooststr. & Reichg., nov. comb.

M. grandiflora Fenzl ex Harv. var. *β* Harv., in Harv. & Sond. Fl. Cap. 3, 1865, p. 166 — *Pentzia intermedia* Hutchinson, in Kew Bull. 1916, p. 249.

Itteren, L., in een verlaten grintgroeve aan de Maas ten N. van het dorp, leg. *J. H. Kern* & *S. J. van Ooststroom*, 20 juni 1959, herb. *v. O.* no. 21438 (herb. L).

Materiaal van deze Composiet werd door ons ter determinatie aan Prof. Dr. Merxmüller (München) ter hand gesteld, die haar met enig voorbehoud als *Pentzia intermedia* Hutchinson determineerde. Later werd zij door collega J. H. Kern, tezamen met de auteur J. Hutchinson vergeleken met het in Kew aanwezige type, waarbij bleek, dat de determinatie van Prof. Merxmüller juist was.

HUTCHINSON (1) geeft een overzicht van het Zuid-Afrikaanse geslacht *Pentzia*, waarin hij o.a. zegt, dat het eigenlijk beter zou zijn, de genera *Pentzia* en *Matricaria* tot één genus te verenigen, maar dat, om zo weinig mogelijk naamsveranderingen te krijgen, het hem toch gewenst toeschijnt, de twee geslachten afzonderlijk te houden. Hij onderscheidt ze als volgt:

Pentzia Thunb.: Hoofdjes zonder straalbloemen; heesters, halfheesters of eenjarige planten. Verspreiding: tropisch en extra-tropisch Zuid-Afrika.

Matricaria L.: Hoofdjes met straalbloemen of bij enkele soorten van het Noordelijk Halfrond zonder deze. Verspreiding: Noordelijk Halfrond, Zuid-Afrika, tropisch Oost-Afrika.

Terwijl wij, door onvoldoende kennis van de Zuid-Afrikaanse soorten, in het midden willen laten of het geslacht *Pentzia* naast

Matricaria al of niet gehandhaafd kan worden, zijn wij toch van mening, dat de door Hutchinson als *Pentzia intermedia* beschreven soort in ieder geval bij het geslacht *Matricaria* moet worden ondergebracht. Er is namelijk geen enkel goed geslachtsverschil aan te wijzen tussen deze Zuid-Afrikaanse soort en b.v. *Matricaria matricarioides*, die op het Noordelijk Halfrond thuishoort, zodat, als wij in deze Hutchinson volgden, deze soorten uitsluitend op grond van hun verschillende geografische verspreiding tot twee verschillende geslachten zouden moeten worden gerekend, wat o.i. niet verantwoord is.

Matricaria intermedia is als volgt gekenmerkt:

Kruidachtige, eenjarige, tot ca. 20 cm hoge plant met vrij dicht behaarde stengels en bladen. Stengel niet of aan de voet vertakt, met opstijgende takken. Bladen dubbel- tot enkel geveerd. Hoofdjes lang gesteeld, alleenstaand aan het eind van stengel of takken, bijna bolvormig, ca. 1–1½ cm in diam., zonder straalbloemen. Omwindselbladen langwerpig lancetvormig, vrij spits, van buiten wollig behaard. Algemene bloembodem gewelfd. Buisbloemen geel, ca. 3½ mm lang, kaal, met in het midden iets ingesnoerde buis en 4 lijn-lancetvormige, spitse, ca. 1 mm lange slippen. Vruchten in omtrek scheef omgekeerd lancetvormig, fijn beklierd. Pappus ontwikkeld als een lage rand.

De soort is alleen bekend van het zuidwestelijke deel van Zuid-Afrika en was tot nu toe nog niet adventief gevonden.

1. J. HUTCHINSON, Notes on African Compositae, III. Kew Bull. 1916, p. 241–254.

77. **Cyperus exaltatus** Retz. (door J. H. Kern, Leiden). (Fig. 3, a-e)

Itteren, L., in een verlaten grintgroeve aan de Maas ten N. van het dorp, leg. *J. H. Kern, S. J. van Ooststroom* & *Th. J. Reichgelt*, 2 sept. 1959, herb. *v. O.* no. 21735 (herb. L); in een verlaten grintgroeve aan de Maas ten Z. van Grevenbicht, leg. *J. H. Kern, S. J. van Ooststroom* & *Th. J. Reichgelt*, 3 sept. 1959, herb. *v. O.* no. 21778 (herb. L).

Het meer dan 600 soorten omvattende geslacht *Cyperus* wordt in een aantal ondergeslachten verdeeld. De omgrenzing van deze ondergeslachten, zoals ze in KÜKENTHAL's monografie wordt gegeven (3, p. 41), is in sommige opzichten kunstmatig, maar voor practische doeleinden meestal zeer bruikbaar.

Bij het subgenus *Cyperus* vallen de rijpe vruchten en hun kafjes acropetaal van de blijvende as van het aartje (de rhachilla) af en zijn de vruchten driekantig. Onze inheemse *Cyperus fuscus* L. behoort ertoe en ook de zelden bij ons aangevoerde *C. eragrostis* Lamk. (6, p. 40 & 41). Van de laatste soort, afkomstig uit Amerika, groeiden in de warme zomer van 1959 talrijke prachtig ontwikkelde exemplaren langs de Maasoever bij Itteren, tezamen met de twee hier besproken *Cyperus*-soorten (zie ook Flor. Not. no. 78), die voordien niet in Nederland werden gevonden.

Ook *Cyperus exaltatus* behoort tot het subgenus *Cyperus* en wel tot de sectie *Exaltati* Kunth, forse, tropische en subtropische planten met

wijd vertakte, samengesteld schermvormige bloeiwijzen, waarin de aartjes in min of meer cylindervormige aren zijn gerangschikt en waarbij de rhachilla gevleugeld is. De soorten van deze sectie zijn onderling zeer nauw verwant, bovendien alle zeer variabel en daarom moeilijk van elkaar te onderscheiden, ook zelfs van de soorten, die door Kükenthal tot de verwante sectie *Fastigiati* Kük. gerekend worden.

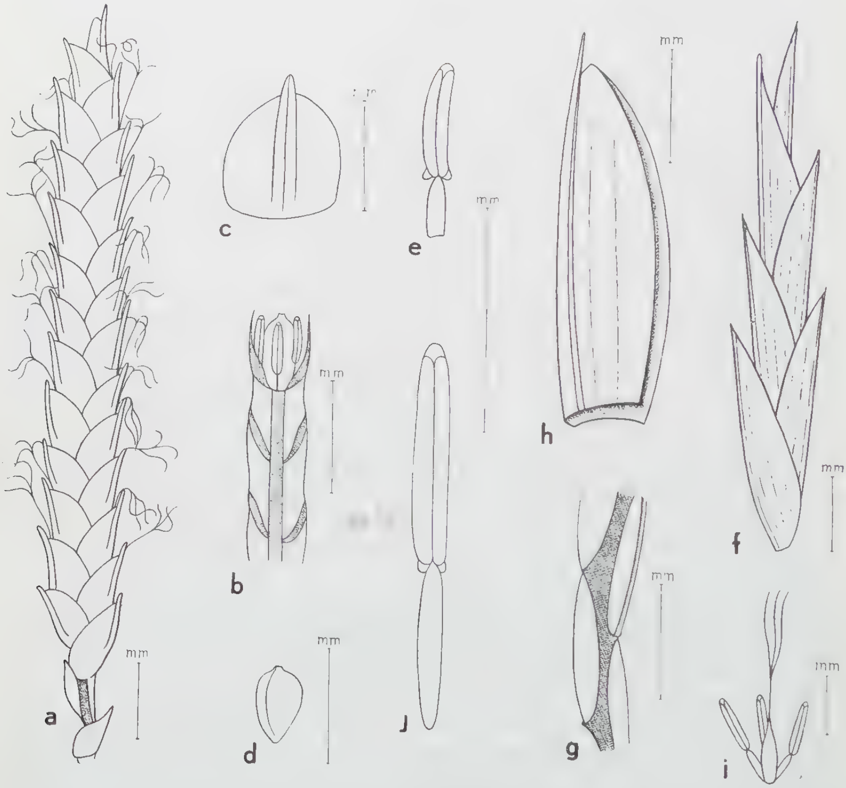

Fig. 3. a–e: *Cyperus exaltatus* Retz., a: aartje, b: rhachilla, abaxiale zijde, c: kafje, d: vrucht, e: meeldraad; f–j: *Cyperus dactyliformis* Boeck., f: aartje, g: rhachilla, laterale zijde, h: kafje, i: bloem, j: meeldraad.

De planten langs de Maas kwamen wel in bloei, maar rijpe vruchten werden niet meer gevormd. Een naar de kassen van de Leidse Hortus overgebracht exemplaar zette in 1960 goed vrucht. Het bleek toen, dat de planten tot *C. exaltatus* behoren en vooral overeenkomen met de vorm van deze soort die in Zuid-Australië voorkomt. Aanvoer met Australische wol is dus zeer waarschijnlijk.

De oorspronkelijke determinatie als *C. imbricatus* Retz. (4, p. 21 en 5, p. 164) bleek onjuist. Zij berustte op het veel minder ontwikkelde bloeiende materiaal, waarbij de aartjes nog kort en de aren vrijwel

zittend zijn. KÜKENTHAL (3, p. 64 & 69) vermeldt, dat bij *C. exaltatus* het aanhangsel aan de top van het helmbindsel rood is, bij *C. imbricatus* wit. Bij de planten uit Limburg is het wit en ook dit leidde tot de foutieve determinatie. Het blijkt echter dat dit kenmerk onbetrouwbaar is. Bij planten uit Zuid-Australië is het aanhangsel ook wit.

Een korte beschrijving van *C. exaltatus* volgt hieronder:

Wortelstok kort, dichte zoden vormend. Stengels driekantig, glad, tot ca. 1 m hoog. Bladen van boven heldergroen, van onderen lichtgroen, met ruwe randen, tot 1 cm breed. Bloeiwijze tot 25 cm lang, de primaire takken tot 15 cm, de secundaire tot 5 cm. Omwindselbladen 4–5, afstaand, tot 80 cm lang. Aren gedeeltelijk gesteeld, vrij dicht tot los, ongeveer 1 cm breed. Aartjes (Fig. 3,a) schuin tot horizontaal afstaand, langwerpig tot lijnvormig, tot 1 cm lang, 1–1 ½ mm breed, veelbloemig. Rhachilla (Fig. 3,b) gevleugeld. Kafjes (Fig. 3,c) schuin afstaand, eirond, stomp, 1 ¼–2 mm lang, met duidelijke stekelpunt. Meeldraden (Fig. 3,e) 3; helmknoppen langwerpig, ⅓–½ mm lang; helmbindsel met kort, glad aanhangsel. Vrucht (Fig. 3,d) ellipsoïdisch of omgekeerd eivormig, met kort topspitsje, geelachtig, ca. ⅔ mm lang, ca. $^2/_5$ mm breed.

De soort is voornamelijk pantropisch, maar bereikt in Australië de staten Victoria en Zuid-Australië. Een afbeelding van een Javaans exemplaar vindt men in Reinwardtia (2, p. 100). Volgens HEGI (1, p. 9) werd de soort in Duitsland en Zwitserland ook enige malen als woladventief aangetroffen.

1. G. HEGI, Illustrierte Flora von Mittel-Europa ed. 2, **2**, 1939.
2. J. H. KERN, Notes on Malaysian Cyperaceae. Reinwardtia **2**, 1952, p. 97–130.
3. G. KÜKENTHAL, Cyperaceae - Scirpoideae – Cypereae. Pflanzenreich IV, **20**, Heft 101, 1935–1936.
4. S. J. VAN OOSTSTROOM & TH. J. REICHGELT, Adventieven langs de Maas in Limburg, II. Natuurhist. Maandbl. **49**, 1960, p. 19–22.
5. ———, Nieuwe plantesoorten in Nederland gevonden hoofdzakelijk in 1959. De Levende Natuur **63**, 1960, p. 161–164.
6. TH. J. REICHGELT, Cyperaceae excl. Carex. Flora Neerl. **1**, afl. 4, 1956.

78. **Cyperus dactyliformis** Boeck. (door J. H. Kern, Leiden). (Fig. 3, f-j)

Itteren, L., in een verlaten grintgroeve aan de Maas ten N. van het dorp, leg. *S. J. van Ooststroom* & *Th. J. Reichgelt*, 18 sept. 1959, herb. *v. O.* no. 21850 (herb. L).

Deze soort behoort tot het subgenus *Mariscus* (Gaertn.) C. B. Clarke, waarbij de kafjes en vruchten aan de rhachilla blijven bevestigd, de aartjes in hun geheel afvallen en de vrucht driekantig is. Van dit ondergeslacht zijn in ons land reeds enkele soorten aangevoerd gevonden, nl. *C. congestus* Vahl, *C. filiculmis* Vahl en *C. cayennensis* (Lamk.) Britt. (3, p. 44–45). *Cyperus dactyliformis* lijkt veel op *C. congestus* en behoort dan ook tot dezelfde sectie, de *Thunbergiani* C. B. Clarke. Ze is meestal minder fors, heeft smallere bladen, kortere aartjes en een smallere, langwerpige vrucht, maar kan vooral onderscheiden worden door de talrijke opvallende dwarsnerfjes waardoor

de nerven in de bladen verbonden zijn. Voorts is de soort als volgt gekarakteriseerd:

Zodevormend. Stengels driekantig, glad, aan de voet verdikt, ca. 30–50 cm hoog. Bladen grijsgroen, aan de top met ruwe randen, 2–5 mm breed; scheden roodachtig bruin. Omwindselbladen 3–5, met ruwe randen, het onderste tot 40 cm lang. Bloeiwijze enigszins samengesteld, met 6–10 takken, die tot 4 cm lang zijn. Aren eibolvormig, met talrijke vingervormig gerangschikte aartjes. Aartjes (Fig. 3,f) langwerpig-lancetvormig, 5–7 mm lang, 1–1½ mm breed, 4–6-bloemig. Rhachilla (Fig. 3,g) breed gevleugeld. Kafjes (Fig. 3,h) langwerpig-eirond, ca. 3 mm lang, iets beneden de top met een spitsje, 7–9-nervig, bleek roodbruin met groene kiel. Meeldraden (Fig. 3,j) 3; helmknoppen lijnvormig; helmbindsel met kort, glad aanhangsel.

De soort is inheems in Zuid-Afrika. De determinatie wordt met enig voorbehoud gegeven, omdat het Limburgse exemplaar nog geen vruchten heeft en de soort mij alleen uit de literatuur (1, p. 329; 2, p. 433) bekend is.

1. O. Boeckeler, Die Cyperaceen des Kgl. Herbariums zu Berlin. Linnaea **36**, 1870, p. 271–512.
2. G. Kükenthal, Cyperaceae – Scirpoideae – Cypereae. Pflanzenreich IV, **20**, Heft 101, 1935–1936.
3. Th. J. Reichgelt, Cyperaceae excl. Carex. Flora Neerl. **1**, afl. 4, 1956.

79. Verwilderde sierplanten

a. **Pulmonaria rubra** Schott

In het bos van Over-Holland, gem. Loenen, Utr., leg. *P. A. Florschütz*, *F. A. Stafleu* & *V. Westhoff*, 2 april 1958 (herb. U).

Deze als sierplant gekweekte soort, met meestal ongevlekte bladen en steenrode, niet naar blauw verkleurende bloemen, werd op genoemde buitenplaats verwilderd aangetroffen. Zij komt oorspronkelijk voor in de Karpaten.

b. **Crocus tomasinianus** Herb.

Ten O. van Oudega, gem. Wymbritseradeel, in een weiland ten NO. van de Skûtelpoel, leg. *Chr. G. van Leeuwen*, 19 maart 1959 (herb. L).

Crocus tomasinianus heeft lila bloemen met een witte keel en spitse bloemdekslippen, die smaller en minder concaaf zijn dan bij *C. vernus* (L.) Hill. Zij is afkomstig uit het noordwestelijke deel van het Balkan-Schiereiland en wordt bij ons als sierplant gekweekt. In genoemd weiland was zij in talrijke exemplaren verwilderd.

SUMMARY

This series of "Floristische Notities" contains with a few exceptions acquisitions to the flora of the Netherlands from the year 1959.

Mentioned are:

68. *Alnus glutinosa* (L.) Vill. f. *quercifolia* (Willd.) Koehne; a case of the occurrence in a wild state of this not rarely cultivated form.

69, 70, 72, 73, 74, 76, 77, and 78. *Dysphania myriocephala* Benth., *Amaranthus macrocarpus* Benth. var. *melanocarpus* Thell., *Glinus lotoides* L., *Verbena supina* L., *Calotis cuneifolia* R.Br., *Matricaria intermedia* (Hutchinson) Van Ooststr. & Reichg.,

nov. comb. (*Pentzia intermedia* Hutchinson), *Cyperus exaltatus* Retz., and *Cyperus dactyliformis* Boeck. First records of these wool-aliens for the Netherlands; all found along the river Meuse near Itteren in the province of Limburg, together with a large number of other adventitious species.

71. *Lepidium pseudo-didymum* Thell. Found for the first time in the Netherlands in 1950 and again in 1958 near wool-factories at Tilburg.

This species was described by Thellung from Galashiels, Scotland, the native country being unknown. It now appears to be identical with *Lepidium inclusum* Schulz from Patagonia and Tierra del Fuego.

75. *Erigeron acer* L. × *E. canadensis* L. (*E.* × *hülsenii* Vatke) was discovered on a railway-yard near Stavoren, where it grew in some quantity together with the putative parents.

79. *Pulmonaria rubra* Schott and *Crocus tomasinianus* Herb., both escaped from cultivation.

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AN ANNOTATED LIST OF MYXOMYCETES FOUND IN THE NETHERLANDS, MOSTLY IN THE VICINITY OF DOORWERTH

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(*Doorwerth*)

(*received October 20th, 1960*)

Since the publication of Dr. T. Broeksmit's paper on Myxomycetes, which includes a list of the representatives of this group then known from the Netherlands (Ned. Kruidk. Arch., 1923: 315–327), and of that by A. M. Scholte (ibid., 1926: 155–162), only a few new finds have been reported.

In July 1953 Dr. W. K. H. Karstens kindly sent me an unpublished list of all the species of which he had seen specimens from the Netherlands; it comprised practically all finds that had been made up to that date, those of myself included. In total 130 species and varieties were enumerated, and of each of them the number of specimens seen by him was recorded. A few of those mentioned by Dr. Broeksmit, viz. *Badhamia nitens*, *Physarum conglomeratum*, *Diderma radiatum*, *Didymium difforme* var. *comatum*, *Lamproderma columbinum*, *Cribraria microcarpa*, *C. splendens*, *Liceopsis lobata* and *Cornuvia serpula* (in the order of the list) are not included in Dr. Karstens' list, either because the specimens were lost, or because the determination proved to be incorrect, and *Arcyria globosa*, one of the eight new indigenes mentioned by Scholte, proved to be *A. cinerea* (private communication). Eighteen species and varieties from Dr. Karstens' list were so far not found by me, whereas my list contains the names of sixty-eight species and varieties not mentioned by him. These species and varieties are marked with an asterisk (*) as they are regarded by me as new to the Netherlands.

I started collecting in August 1951, mostly in the vicinity of Doorwerth, and unless another locality is mentioned, the specimens were all collected in the area between Renkum and Oosterbeek, Wolfheze and the Rhine; the notes on the frequency apply to this area alone.

Quite a number of my Myxomycetes were obtained in "culture", i.e. on pieces of bark, either from dead or from living trees, or on fragments of dead wood that were soaked for a few hours in tap water, then placed in a dish on wet blotting paper and covered with plastic, the dish subsequently being kept at room temperature. Myxomycetes usually develop after a week or a fortnight, although some require about a month (e.g. some species of *Licea*).

The colour of the plasmodium is recorded only when the plasmodium was seen by myself; it is not necessarily the same as that given in the monographs.

The names are those used by Dr. G. W. Martin in the North

American Flora, Vol. 1, part 1 (1948); where they differ from those used in the well-known monograph by the Listers, the name applied by the latter is added between brackets. These names are also mentioned in the alphabetical sequence, but there they are written in small type with a reference to the name used by Martin.

In due time I hope to describe my specimens more fully, citing the numbers of the collections; a start has already been made in "Notes on Myxomycetes IV" published elsewhere in this journal.

Amaurochaete cribrosa Sturgis cf. *A. tubulina*

Am. fuliginosa (Sow.) Macbride
On practically undecayed coniferous wood; common. Plasmodium white, then carmine tinted, turning deep mulberry red, and finally black.

Am. tubulina (Alb. et Schw.) Macbride (*A. cribrosa* Sturgis)
In the same habitats as the preceding species, and often simultaneous with it; five collections. Plasmodium too like that of the preceding species.

Arcyodes incarnata (Alb. et Schw.) O. F. Cook (*Lachnobolus congestus* Lister)
On decaying wood; not rare. Plasmodium white.

* *Arcyria carnea* G. Lister
On decaying wood; not rare. Plasmodium white.
This species has apparently often been confused with others, especially with *A. insignis, A. denudata* and *A. incarnata*; in colour and size it is similar to an unexpanded *A. incarnata.* The capillitial net, however, has very fine meshes, a character which permits identification by the aid of a hand lens; further it is impossible to blow the firmly attached capillitium out of the cup. Various authors mention a close resemblance to *A. cinerea*, but this has never struck me.

Arc. cinerea (Bull.) Pers.
On decaying wood; very common. In dense colonies or scattered; in cultures usually scattered; either nearly white, dirty yellowish, beige, greenish, bluish or grey. Plasmodium white.

Arc. denudata (L) Wettst.
On decaying wood, rarely on leaves, but then usually near decaying wood; common. Plasmodium white.

Arc. ferruginea Sauter
Mostly on oak wood, on decaying stumps as well as on a living tree; on the latter about one meter from the ground; not rare. Plasmodium pink or salmon.

Arc. incarnata (Pers.) Pers. var. *incarnata*
On decaying wood, especially on fallen branches; very common. Plasmodium white.

* *Arc. incarnata* (Pers.) Pers. var. *fulgens* Lister
This variety is described as dark reddish brown and provided with firm stalks. As no mention is made of the capillitium,

we may assume that this is similar to that of the type. If this surmise is correct, then I have collected the variety a few times only. However, a large number of specimens to which the description of the colour and of the stalks is applicable, cannot be placed here because of the capillitium, which has much smaller meshes, and expands therefore less strongly, of the cup, which is asymmetrical, and of the structure of the capillitium threads, which are thicker, more densely spinulose and partly reticulate. They represent apparently an undescribed species. Pending further study, I will mention it below as *A.* spec.

Arc. nutans (Bull.) Grev.
On dead wood, and sometimes on living trees; very common. Plasmodium white.

Arc. oerstedtii Rost.
On decayed beech and pine wood; once on a stump of an unknown tree; not rare. Pasmodium white.

Arc. pomiformis (Leers.) Rost.
On decayed wood; very common. Plasmodium white.

* *Arc.* spec.
See above under *A. incarnata* var. *fulgens*. Plasmodium white.

Badhamia affinis Rost. var. *orbiculata* G. Lister cf. *B. orbiculata*

Badh. foliicola Lister
On grass, dead leaves and pine needles; common; in some years in spectacular quantities. Plasmodium pale or deep yellow.

* *Badh. lilacina* (Fries) Rost. var. *lilacina*
Collected at Kortenhoef in a Sphagnum bog and on the brink of the small islands found in the large sheet of water there, where it proved to be common. The tufts of sporangia vary in colour from ash-grey to dusty rose. Plasmodium (of a specimen found on one of the islands) yellow.
I have marked this find with an asterisk, although the name occurs in the list of Dr. Karstens. Here, however, it was applied to a specimen collected by me at Ootmarsum, for which it can not be retained, as it is the type of my var. *megaspora*. The specimens collected in 1958 at Kortenhoef are therefore the first that are known from the Netherlands.

* *Badh. lilacina* (Fries) Rost. var. *megaspora* N.E.N–B. (Acta Bot. Neerl. **7**: 184. 1958)
Known only from the marshy source of the "Poelbeek", Ootmarsum.

Badh. macrocarpa (Ces.) Rost.
Two collections only, one from the bark of a recently felled beech, the other from a dead fruit tree. Plasmodium white.

* *Badh. orbiculata* Rex (*B. affinis* Rost. var. *orbiculata* G. Lister)
On fallen bark of dead fruit trees; a few collections only. Plasmodium white.

Badh. panicea (Fries) Rost.

On the bark of dead trees and on stumps; common. Plasmodium white.

Badh. utricularis (Bull.) Berk.

On dead wood, bark, Stereums and even on sand; common. Extremely variable in all its properties. Plasmodium deep yellow to orange.

* *Badh. viridescens* Meylan

Up to now known from Switzerland and Scotland only. Obtained once in culture from a yellow plasmodium on bark of a dead fruit tree; the collection consists of seven well developed sporangia and a few abnormal ones.

Brefeldia maxima (Fries) Rost.

My attention was asked for a large, white plasmodium on the Hemelse Berg near Oosterbeek by Dokter J. H. Broers, who had found it there the previous year too. According to his description there was such a quantity of plasmodium then, that it looked as if buckets of whitewash has been overturned and splashed about; later the plasmodium had moved up the tree trunks and the stems of herbaceous plants, where the sporangia developed in some parts a meter above the ground. Also collected by E. T. Nannenga on a large stump of Populus at Rijnauwen near Utrecht.

Ceratiomyxa fruticulosa (Mull.) Macbride

On decaying wood; very common. Very variable in shape and colour. Plasmodium watery and white, ivory, salmon or rose.

* *Cienkowskia reticulata* (Alb. et Schw.) Rost.

On the bark of a fallen beech branch; collected only once. Plasmodium orange.

* *Clastoderma debaryanum* A. Blytt

On decaying oak wood piled up to be used as firewood; collected only once. Plasmodium watery white.

* *Colloderma oculatum* (Liert) G. Lister

Difficult to detect in the open, where I collected it once on moss on a living oak. Obtained in culture from mossy bark of birch. Plasmodium dark and opaque, brown.

* *Comatricha cornea* G. Lister et Cran

On bark of elm, only in culture. Plasmodium watery white.

Com. elegans (Racib.) Lister var. *elegans*

On decaying wood; not rare. Plasmodium watery white.

* *Com. elegans* (Racib.) Lister var. *palens* G. Lister

On decaying pine wood; once collected.

* *Com. fimbriata* G. Lister et Cran

On bark and wood of dead and living trees; very common. Because of its small size it is easily overseen, but it may be found by turning fallen branches against the light, as then they stand out against the light background. Appears in most cultures. Plasmodium watery white.

Com. flaccida (Lister) Morgan (*Stemonitis splendens* Rost. var. *flaccida* Lister)
On coniferous stumps; common. Plasmodium white, pale yellow or yellow.

* *Com. irregularis* Rex ?
One specimen from a decaying beech stump seems to belong here; if the identification is correct, it is the first record for Europe. Plasmodium white.

Com. laxa Rost. var. *laxa*
On decaying wood, common. Plasmodium watery white.

* *Com. laxa* Rost. var. *rigida* Brandza
On dead wood; a few collections only.

* *Com. longa* Peck
Recorded from Europe up to now only from a glass-house near Berlin and from Roumania. Thrice collected by me; twice on the same beech stump, in consecutive years; once from another beech stump, a few kilometers from the first.

Com. nigra (Pers.) Schroet. var. *nigra*
On wood and bark; very common. Very variable. Plasmodium watery white.

Com. nigra (Pers.) Schroet. var. *alta* Lister
On fallen branches of lime and beech; a few collections only. Plasmodium watery white.
This variety is easily recognizable because the free ends of the capillitium threads in the basal part of the sporangium are distinctly thickened (clubshaped), and because the capillitium forms a flimsy tangle, which, when the sporangium dehisces, expands in one direction to as much as ten times its original height.

Com. pulchella (C. Rab.) Rost. var. *pulchella*
On dead leaves and grass; very common. It is one of the few species which occur regularly on dead bracken leaves. Plasmodium watery white.

* *Com. pulchella* (C. Rab.) Rost. var. *gracilis* Lister
Collected once on a decaying beech stump covered with grass and dead leaves.

Com. tenerrima (M. A. Curt.) G. Lister
On old straw and on fallen branches; especially common on old straw. Plasmodium watery white.

Com. typhoides (Bull.) Rost.
On decaying wood; very common. Plasmodium watery white.

* *Com.* spec.
Close to *C. acanthoides* Alexopoulos (Mycologia **50**: 55. 1958), but differing in the denser capillitium and in the spores, which are not spinose but warted. Truly "solitary" indeed; the three sporangia in my collection were obtained in culture, each from the bark of another kind of tree. Plasmodium watery white.

Craterium aureum (Schum.) Rost.
On dead leaves and grass; in some summers common. Plasmodium yellow.

* *Crat. auronucleatum* N.E.N–B. (Acta Bot. Neerl. **10**: 62. 1961)
Found only once, on dead leaves and living grass.

* *Crat. concinnum* Rex
Found only once, on a dead leaf in a ditch under bracken, Bilthoven. First record for Europe (cf. Acta Bot. Neerl. **10**: 61. 1961).

* *Crat. cylindricum* Massee (*C. leucocephalum* (Pers.) Ditmar var. *cylindricum* Lister)
Found only once (leg. J. L. Nannenga) on a cap of a milk-bottle between dead leaves.

Crat. leucocephalum (Pers.) Ditmar var. *leucocephalum*
On dead leaves and on piles of garden refuse; common. Plasmodium yellow.

Crat. leucocephalum (Pers.) Ditmar var. *cylindricum* Lister cf. *C. cylindricum*

* *Crat. leucocephalum* (Pers.) Ditmar var. *scyphoides* Lister
On dead leaves and stems of herbaceous plants; collected a few times in the same site as the typical form. Plasmodium yellow.

Crat. minutum (Leers) Fries
On dead leaves and other herbaceous matter; common. Plasmodium yellow.

Cribraria argillacea (Pers.)Pers.
On decaying coniferous wood; common. Plasmodium dark grey or blue-grey.

Crib. aurantiaca Schrad.
On decaying coniferous wood; very common. Plasmodium bright green.

* *Crib. ferruginea* Meylan
Collected only once, viz. on a mossy pine stump near Leersum.

Crib. intricata Schrad. ?
With considerable misgivings I place here, at least provisionally, a large number of collections of a small, nutbrown Cribraria. Their size is about two thirds of that of *C. aurantiaca*. The cup, seen by the aid of a hand lens, looks almost smooth, but under the microscope it proves to be minutely striate with striae radiating from the stipe; it is moreover provided with very delicate concentrical or more or less spiral wrinkles; its height is half to one third that of the sporangium as a whole. The nodes are large and flat; the granules of the same size as those of *C. aurantiaca* and *C. intricata*, and pale, rarely dark. The threads by which the nodes are connected with each other, do not run parallel as in *C. intricata*, but there is, as a rule, more than one connection between the nodes; some of the threads, moreover, have free ends. The spores are about 8 μ in diam., minutely spinulose and also provided with warts, of which in the optical section five or six are seen on the circumference; these warts are often connected by faint rows of spinules, and then the surface of the spores looks very laxly reticulate. Plasmodium dark grey.

* *Crib. macrocarpa* Schrad.

Once collected on a practically undecayed fallen pine branch and on the needles of the latter. "De Pan" near Zeist.

* *Crib. microcarpa* (Schrad.) Pers.

Once collected on a decaying stump, where on account of its small size it is easily overlooked. In culture it appeared on the inner side of the bark which was pealing off from a dead oak branch and on coniferous wood (also on coniferous wood taken home from Bilthoven). Plasmodium grey, turning hyaline when the very long stalks of the sporangia have been formed. At that time too the nodes make their appearance, and as the sporangium is at this stage still hyaline, they stand out very conspicuously.

Crib. rufa (Roth) Rost.

On decaying coniferous wood; common; one collection from an oak stump. Plasmodium sometimes dark grey, turning dirty white, but more often already dirty white when emerging from the wood.

Diachea leucopodia (Bull.) Rost.

On dead leaves, bramble stems, living grass and herbs; not rare. Also one collection from Elst in the Betuwe, where it was found for several weeks in considerable quantity on a heap of garden refuse, and two collections received from Dr. G. H. Boerema, who collected them on grass and on strawberry leaves at Wageningen. Plasmodium white.

* *Dianema harveyi* Rex

Collected several times but always in the same locality, where it occurred on fallen branches of a lime tree. Plasmodium pink.

Dictyaethalium plumbeum (Schum.) Rost.

On fallen branches; not rare. In one aethalium collected on a dead branch of Prunus laurocerasus the spore mass has a rusty colour, not the usual yellow or ochraceous tint. Plasmodium pink; in the aberrant specimen not seen.

Dictydium cancellatum (Batch) Macbride var. *cancellatum*

On decaying wood; common. Plasmodium black; the wood from which it emerges, sometimes stains purple.

Dictyd. cancellatum (Batch) Macbride var. *fuscum* Lister

On decaying wood; common. Plasmodium black.

Diderma effusum (Schw.) Morgan

On dead leaves; not rare. Plasmodium white.

* *Dider. deplanatum* Fries

Collected in one place only, where it returns every winter; it grows here on dead oak leaves. Plasmodium white.

Dider. floriforme (Bull.) Pers.

Only once collected, on a beech stump.

Dider. globosum Pers.

Collected only once, on dead leaves under a hazel shrub. Plasmodium white.

Dider. hemisphaericum (Bull.) Hornem.
On dead leaves, twigs and refuse of herbs; not rare. Plasmodium white.

Dider. montanum (Meylan) Meylan
Collected only once on a small mossy stump.

* *Dider. radiatum* (L) Morgan var. *umbilicatum* Meylan
Regularly collected at two sites, where it occurred on dead twigs and on living moss. Rather similar to *D. montanum* and rather unlike *D. radiatum* var. *radiatum*. Plasmodium white.

Dider. spumaroides (Fries) Fries
Only two collections, one with small, scattered, grey sporangia on a hardly visible hypothallus, and one with strongly congested white sporangia inserted on a crustose, white hypothallus; both from leaves. Plasmodium of the specimen with grey sporangia white.

Dider. testaceum (Schrad.) Pers.
On dead leaves and bramble stems, sticks and living grass and herbs; not found before 1960, but then locally abundant. Plasmodium whitish.

* *Dider.* spec.
A small collection of pale rose sporangia provided with an angular stipe and a small and globose pink columella. I have as yet not succeeded in placing it.

Didymium anellus Morgan
Collected only once, on a pile of herbaceous stems thrown away in a wood.

Didym. clavus (Alb. et Schw.) Rab.
On dead leaves and on bark of living trees; appearing also in cultures; common. Plasmodium grey or dirty white.

Didym. complanatum Rost. cf. *D. serpula*

Didym. difforme (Pers.) S. F. Gray var. *difforme*
On decaying vegetable matter; very common. Plasmodium dirty white.

* *Didym. difforme* (Pers.) S. F. Gray var. *comatum* Lister
Here I place a number of specimens which differ considerably from *D. difforme* var. *difforme*, not only in the more profuse capillitium and in the paler spores, the characters mentioned in the description of the variety, but also in the closely adherent sporangium walls, the white or nearly white and rugose base of the sporangium, the very weak attachment of the capillitium to the base of the sporangium as well as to the peridium, and the subglobose, minutely reticulate spores. On account of the weak attachment of the capillitium, as soon is the lid is off, the latter comes out, in the plasmodiocarpous forms as a woolly mass, in the sporangiate ones in the form of a column which may reach a height of about ten times the diameter of the sporangium. If my specimens really are identical with those of the Listers (I did not like to touch any of the very few sporangia in the Lister collection in the British Museum),

this variety would, in my opinion, deserve specific rank. Rests the question whether *Chondrioderma calcareum* Rost., which has been quoted as a synonym, is actually identical. As the type of this species does not seem to be available, it will be difficult to answer this question.

* *Didym. difforme* (Pers.) S. F. Gray var. *repandum* Lister
Three collections, on dead remains of herbs.

* *Didym. dubium* Rost. (*D. wilczeki* Meylan)
Five collections only of which two are limeless, on dead herbaceous plants. May be this mainly mountainous species may be considered a relic from glacial times, like some hepatics occurring in this neighbourhood.

Didym. iridis (Ditmar) Fries (*D. nigripes* Fries var. *xanthopus* Lister)
On dead leaves and grass (n. 32 was identified for me by Dr. Karstens).

Didym. megalosporum Berk. et Curt. (*D. nigripes* (Link) Fries var. *eximium* Lister)
On leaves, fallen pine needles and stems of herbaceous plants; not rare. Plasmodium yellow.

Didym. melanospermum (Pers.) Macbride
On fallen leaves, pine needles, grass, herbs and moss; common. Plasmodium dirty white.

Didym. minus (Lister) Morgan
In similar habitats as the preceding species; not rare.

Didym. nigripes (Link) Fries var. *nigripes*
On dead leaves, living grass and ivy; common. Plasmodium brown.

Didym. nigripes (Link) Fries var. *eximium* Lister cf. *D. megalosporum*
Didym. nigripes (Link) Fries var. *xanthopus* Lister cf. *D. iridis*

* *Didym. ovoideum* N.E.N–B. (Acta Bot. Neerl. **7**: 780. 1958)
The type was collected at Bilthoven in a ditch under oak and bracken, where it yearly returns; another specimen is from Doorwerth on pea straw. Plasmodium yellow.

* *Didym. serpula* Fries (*D. complanatum* Rost.)
On dead leaves, pine needles and living grass; four collections. Plasmodium yellow.

Didym. squamulosum (Alb. et Schw.) Fries
On dead leaves, etc.; very common. Very variable in all its parts. Plasmodium pure or dirty white.

* *Didym. trachysporum* G. Lister
Collected once, on a pile of decaying potato plants in a beech plantation, but next to a potato field.

* *Didym. vaccinum* (Dur. et Mont.) Buchet
On tightly packed straw decaying in the garden, where it appeared continuously for some weeks. Plasmodium orange.

Didym. wilczeki Meylan cf. *D. dubium*.

* *Echinostelium fragile* N.E.N–B. (Acta Bot. Neerl. **10**: 65. 1961)
Known only from a culture of bark from a living horse-

chestnut tree on the "Hemelse Berg" near Oosterbeek. Plasmodium watery, pale pink.

* *Ech. minutum* De Bary

Because of its minute size not likely to be found in the open, but very common on wood and bark in cultures. Mostly white, but pale pink and beige sporangia are also met with. Plasmodium watery.

Enerthenema papillatum (Pers.) Rost.

On dead wood; common. It appears also regularly in cultures on bark taken from living trees, and occurs in two forms, viz. one with fine, smooth, only slightly branched capillitium, and one with thick, black, much branched and spinulose capillitium. Plasmodium watery white.

Enteridium olivaceum Ehrenb.

Not rare on logs and fallen branches. Plasmodium pink. About one half of my specimens have the spores clustered, and then the latter are more spinose on the free side; in the other half the spores are free, and then the spinules are evenly distributed over the surface.

* *Fuligo cinerea* (Schw.) Morgan

Found only once, on dead leaves and pine needles.

Ful. septica (L) Weber var. *septica*

Macbride and Martin quote in their monograph several varieties of *F. septica*. In the following I will cite their diagnoses between quotation marks. The typical form is said to be "large, yellow. ochraceous or tawny, with an extremely friable, foamy cortex. The calcareous internal structure is white. The spores are spherical, nearly smooth and 6–8 μ in diam". This typical form is not common in this neighbourhood; most yellow or yellowish Fuligos tend to bleach, whereas the internal structure is usually pure yellow, and these, therefore, belong to the var. *flava*.

* *Ful. septica* (L) (Weber) var. *flava* Pers.

"Aethalia wide-spread, but thin, yellow or yellow green, the cortex yellow but extremely evanescent; the capillitium yellow throughout. The spores tend to average somewhat larger than those of the other varieties, 8–9 μ. On fallen logs in moist dark woods". This is the most common form; it is found on tree stumps, grass and dead leaves. Plasmodium yellow.

Ful. septica (L) Weber var. *candida* Pers.

"Plasmodium, aethalium and limeknots white, giving the whole structure a grayish white appearance when mature". This form too is common, and is found in the same habitats as the var. *flava*. Plasmodium white, rarely creamy.

Ful. septica (L) Weber var. *rufa* Pers.

"Aethalium usually thick and rather solid; much less flattened in proportion than the typical form; the cortex is porose but firm, orange at first, becoming tawny with age, often exhibiting

two distinct layers; the interior grayish; the spores 6–7 μ. Forest". Common on stumps, less common on grass: often forming extensive aethalia. Plasmodium yellow.

* *Ful. septica* (L) Weber var. *laevis* Pers.

"Similar to the preceding, but usually smaller, with a smooth, rusty brown, persistent cortex, which remains when all the grayish sporiferous mass has been distributed. Forest". Only once collected.

Hemitrichia clavata (Pers.) Rost.

Two collections only; one from a decaying beech stump, the other from dead wood in the trunc of a living ash.

Hem. karstenii (Rost.) Lister?

On bark of various dead trees; not rare. Plasmodium white. This is generally considered to be no more than a form of *Trichia contorta*; it may be that my specimens are T. contorta, as the capillitial net is not very pronounced.

* *Hem. leiotricha* Lister

Two collections only; the first, consisting of two sporangia, from the petiole of a birch leaf, the second, consisting of a few more sporangia, from a dead leaf.

Hem. minor G. Lister cf. *Perichaena minor*

* *Hem. stipitata* (Massee) Macbride

Two collections only, both on beech stumps; one by M. A. Nannenga.

Hem. vesparium (Batsch) Macbride

On decaying tree stumps; very common. Plasmodium black. In my specimens the capillitium is but rarely branched, and this species would therefore, in my opinion, better be placed with *Trichia*.

Lachnobolus congestus Lister cf. *Arcyodes incarnata*

Lamproderma arcyrioides (Sommerf.) Rost. (*L. violaceum* Rost.)

Collected only once; on a decaying beech stump in winter.

* *Lamp. arcyonema* Rost.

One collection only, from an oak stump near Utrecht (leg. E. T. Nannenga).

Lamp. scintillans (Berk. et Br.) Morgan

On dead leaves, twigs and pine needles and on living ivy petioles; very common. Plasmodium watery white.

Lamp. violaceum Rost. cf. *L. arcyrioides*

Leocarpus fragilis (Dicks.) Rost.

On various substrates; common. Plasmodium yellow, often becoming orange as the sporangia are taking shape.

Mostly with free spores measuring from 9 to 13 μ in diam.; in two collections the spores are circ. 17 μ in diam., whereas in one collection (Groningen, leg. E. A. Agsteribbe) they are clustered.

* *Licea biforis* Morgan

Obtained twice from bark of living trees in cultures; viz. once from that of a beech near the "Plasmolen", Mook, and once from that of a horse-chestnut on the "Hemelse Berg" near Oosterbeek. With these findings the Netherlands become the third country in Europe from which this species is known: Rostafinsky recorded it for Poland, Alexopoulos (Brittonia **10**: 26. 1959) for Greece. Plasmodium dark opaque brown.

* *Lic. castanea* G. Lister

Collected several times during the winter in the same place, on the bark of a felled elm, mostly on its inner side; also on bark of this tree in culture. Plasmodium yellow-brown.

Lic. flexuosa Pers. cf. *L. variabilis*

Lic. minima Fries

In most cultures in due time small black Liceas appear. Whether all of these may be referred to *L. minima* and to *L. pusilla*, is dubious. However, there are in my collection two groups either of which may perhaps be placed in *L. minima*. The first consists of specimens from decaying pine wood; their spores are pale red-brown and about 12 μ in diam. This seems to agree well with the original description of Fries, who called them "rubiginose". Lister describes the spores as olivaceous-brown or lilac-brown, but he does not say in what condition he studied them. The red spores of the specimens of this group turn olivaceous in dilute KOH, and this may have been the condition in which they were studied by Lister. The second group was obtained from the bark of dead fruit trees; here the spores are grey, and measure circ. 13 μ in diam., and they are rather coarsely warted, whereas those of the first group are merely spinulose, and the peridium fragments are not undulate or red-brown as in the latter, but flat and yellow-brown. The exact position of these taxa will be difficult to determine, but provisionally they may be placed under *L. minima*. They were collected a few times in the open, but on account of their small size, they are easily overlooked.

* *Lic. operculata* (Wingate) G. W. Martin (*Orcadella operculata* Wingate)

Obtained several times from bark of the same lime tree in culture. Plasmodium dirty yellow.

* *Lic. parasitica* (Zukal) G. W. Martin

Appears regularly on bark from living trees in cultures. Plasmodium yellow-brown, gelatinous.

* *Lic. pusilla* Schrad.

Described as being chestnut-brown with large spores which are very dark in mass, and as appearing on coniferous wood. In cultures on decaying coniferous wood I obtain regularly small black Liceas to which this description applies very well. The spores are about 17 μ in diam., and dark brown. The sporangium wall is dark with included matter, but yellowish brown by transmitted light. However, the undulate edges described by Lister and by Hagelstein for *L. pusilla* are absent

in the specimens I myself place here, whereas they are a constant feature of one of the groups of specimens I have provisionally placed under *L. minima*. The question of the identity of the specimens described by Fries and Schrader will be difficult to decide!

Lic. variabilis Schrad. (*L. flexuosa* Pers.)
In autumn and winter on decaying coniferous wood; common. Plasmodium pinkish, turning yellowish.

* *Lic.* spec.
The minute sporangia of this species were obtained in culture on bark of living elm, lime and oak covered by moss. It resembles to some extent *L. fimicola*, but the sporangia are brown, strongly depressed, oval in outline and provided with a dark rim of granular matter round the base. The number of sporangia appearing at a time is but small. Comparison with the type of *L. fimicola*, kindly sent by Dr. G. W. Martin, proves the two to be distinct: the spores, although smooth or nearly so, are appreciably smaller in my specimens.

Lycogala conicum Pers.
Collected three times on decaying beech stumps, twice in the vicinity of Doorwerth and once at the "Plasmolen" near Mook; the last-mentioned specimen, unfortunately, has been lost. Plasmodium pink.

Lyc. epidendrum (L) Fries
On decaying wood; very common. Plasmodia either orange-red to vermilion or carmine; in the first case the aethalia become beige, in the second dark grey-brown.

Lyc. flavofuscum (Ehrenb.) Rost.
On rotting wood parts in living trees, especially in beech (one collection from birch); not rare. Plasmodium white.

* *Lyc.* spec.
At Doorwerth once a small aethalium was found on pine needles. The cortex was very thin, transparent and evanescent; the pseudocapillitium consisted of tubules with a rather narrow lumen and with large constrictions; the spores were in mass as well as by transmitted light pale rose. The material does not seem sufficient for the description of a new species, but perhaps more may be found.

Margarita metallica (Berk.) Lister var. *metallica*
Once a few sporangia were found on grass, but it often appears on bark in cultures, especially in winter. Plasmodium pink or white.

* *Marg. metallica* (Berk.) Lister var. *plasmodiocarpa* R. E. Fries
Collected once on the bark of a living horse-chestnut at Schaloen, South Limburg. Plasmodium pink.

Mucilago spongiosa (Leysser) Morgan
Twice collected by E. T. Nannenga on grass, once on a roadside at Doorwerth, and once from the base of a dike at

Elden, in the Betuwe. From Dr. G. H. Boerema I received two specimens collected at Wageningen, also on grass.

* *Oligonema flavidum* (Peck) Peck

On a decaying beech stump in an exposed position, where it returned for several years.

Orcadella operculata Wingate cf. *Licea operculata*

Perichaena corticalis (Batsch) Rost.

On bark of dead trees; not rare. Plasmodium grey.

Per. depressa Lipert

On bark of dead trees; not rare. Plasmodium wine-red.

* *Per. minor* (G. Lister) Hagelstein (*Hemitrichia minor* G. Lister)

On decaying vegetable matter, twigs and branches; not rare.

Per. vermicularis (Schw.) Rost.

On decaying vegetable matter; not rare.

Physarum auriscalpium Cooke

Two small collections from nearly the same spot; on bark fallen from a dead fruit tree.

Phys. bethelii Macbride (*P. viride* Pers. var. *bethelii* G. Lister)

On decaying pine stumps; common; one collection from Bilthoven. Plasmodium yellow.

Most of my specimens have longer stalks than the type specimen; however, I have also specimens in which the sporangia are partly sessile and partly stalked, with every gradation between. The species is nevertheless easily recognizable, as it has a facies of its own, viz. a dull gloss, a dimple on the top, and umbilicate at the base, all very striking features. It seems to have been collected but rarely; there are only three specimens in the Lister collection, none, as far as I know, in that of Howard (at least not in the part which is in the British Museum), and in America the situation is much the same, i.e. also three collections at the University of Iowa. It was known so far only from Colorado, Washington and Roumania; so that this is the second record for Europe. One of my specimens was identified by Dr. Karstens, and included in his list, although with a question mark; later Dr. G. W. Martin studied my number 1634, and referred it to this species; he has remeasured the spores of the type, and found them to be 12.5–13.5 μ in diam.; those of my specimen are about 12 μ. I would like to thank here both Prof. Karstens and Prof. Martin for their kind help.

Phys. bitectum G. Lister

On dead leaves and straw; not rare. Plasmodium white.

Phys. bivalve Pers. (*P. sinuosum* Weinm.)

On dead leaves and twigs; not rare. Plasmodium white.

Phys. cinereum (Batsch) Pers.

On dead leaves, living grass and herbs; common. Abundant in some summers. Very variable. Plasmodium usually white, sometimes pale yellow.

Phys. citrinellum Peck cf. *P. flavidum*

Phys. compressum Alb. et Schw.

On straw and on rotting vegetable matter in general; common. Plasmodium white.

* *Phys. confertum* Macbride

Twice collected; once on dead leaves, and once on living grass. Plasmodium pale yellow.

Phys. connatum Lister cf. *P. notabile*

Phys. contextum (Pers.) Pers.

On dead leaves and sticks; not rare. Nearly white, yellow and orange. Plasmodium yellow.

* *Phys. didermoides* (Pers.) Rost.

A specimen was given to me by Dr. J. C. Sobels, who obtained it in culture from spores from a collection made by Dr. P. S. J. Schure near Leiden.

* *Phys. didermoides* (Pers.) Rost. var. *lividum* Lister

Twice collected, once on rotting potato stems, the other time on pine needles.

* *Phys. flavidum* (Peck) Peck (*P. citrinellum* Peck)

Once collected on a dead leaf, where it occurred in company of *Craterium aureum*. Plasmodium yellow.

* *Phys. lateritium* (Berk. et Rav.) Morgan

Collected once on dead oak leaves.

Phys. leucophaeum Fries (*P. nutans* Pers. subsp. *leucophaeum* Lister)

On dead wood; very common. Plasmodium greyish-white.

* *Phys. mucosum* N.E.N–B. (Acta Bot. Neerl. **7**: 782. 1958)

Twice collected, on dead leaves and sticks. Plasmodium yellow.

* *Phys. murinum* Lister

Especially on pine wood; not rare. Plasmodium opaque, pale yellow.

The specimens I place here, are in fact more or less intermediate between *P. globuliferum* (Bull.) Pers. and *P. murinum*. In my specimens the peridium is rich in pale fawn or beige coloured lime scales, the lime-knots are pale yellowish-brown, and the stipe is short. In the Lister collection I came across a specimen (BM 2101) which resembled those of mine; it was placed with *P. globuliferum*, but with the note: "near *P. murinum*".

Phys. notabile Macbride (*P. connatum* Lister)

Once collected; on a tree stump.

Phys. nutans Pers. var. *nutans*

On wood, leaves and pine needles a slender long-stalked form is found, which shows a strong resemblance to *P. viride*, but which is slightly larger and without any trace of yellow; on wood a second form is found in which the sporangia are more nearly globose and provided with a shorter stalk, resembling the figure which in Lister is given for the species (3rd ed. Fig. 37d); very common. Plasmodium grey, turning white or pale yellow as the sporangia are taking shape.

Phys. nutans Pers. var. *leucophaeum* Lister cf. *P. leucophaeum*

Phys. nutans Pers. var. *robustum* Lister

On dead wood; very common. Plasmodium grey, turning white or pale yellow as the sporangia are formed.

Phys. psittacinum Ditmar

Only once collected, on the bark of a fallen oak branch. Plasmodium orange.

Phys. sinuosum Weinm. cf. *P. bivalve*

Phys. straminipes Lister ?

Collected only once, on a box of rotting cherries from the year before. Plasmodium white.

The spores are very dark and distinctly facetted, and measure 13–14 μ in diam., which is rather large for this species, and the capillitium is badhamioid, which also looks unusual.

Phys. vernum Fries

On straw, hay and dead leaves; a few collections only. Plasmodium white.

Phys. virescens Ditmar

On moss, grass and leaves in open woods; common. Plasmodium yellow.

Phys. viride (Bull.) Pers. var. *viride*

On dead wood and twigs; very common. Plasmodium yellow.

Phys. viride (Bull.) Pers. var. *aurantium* (Bull.) Lister

On dead wood; common. Plasmodium orange.

Phys. viride (Bull.) Pers. var. *bethelii* G. Lister cf. *P. bethelii*

* *Reticularia intermedia* N.E.N–B. (Acta Bot. Neerl. **7**: 773. 1958)

Collected a few times on dead beech branches, "Boersberg", Doorwerth.

* *Ret. jurana* Meylan (*R. lycoperdon* Bull. var. *jurana* G. Lister)

On decaying wood; very common. Plasmodium first white, then rose, beige and dark brown; the rose stage but rarely evident.

Ret. lycoperdon Bull. var. *lycoperdon*

On dead wood; common. Plasmodium white.

Ret. lycoperdon Bull. var. *jurana* G. Lister cf. *R. jurana*

Stemonitis axifera (Bull.) Macbride (*S. ferruginea* Ehrenb.)

On dead wood; very common. Plasmodium white.

Stem. confluens Cooke et Ellis

The description of this species given by the Listers and that given by Macbride and Martin differ in various points. My own specimens, all from bark pealing off from a dead fruit tree, and mostly from the inner side of the bark, agree very well with the description given by Macbride and Martin, for they are sooty black and provided with very dark spores, measuring 13–14 μ in diam. Although I have been on the lookout for the plasmodium, and have gathered the species time and again, I have never seen it. According to Lister as

well as to Macbride and Martin it would be white, but I can hardly believe that the specimens collected by me can have shown such a conspicuous tint in any stage.

Stem. flavogenita Jahn

On dead wood, and sometimes on twigs and dead leaves; common. Plasmodium yellow.

Stem. fusca Roth var. *fusca*

On dead wood; very common. Very variable. Plasmodium white.

Stem. fusca Roth var. *confluens* Lister

On dead wood, thick fallen branches and tree stumps; not rare. Plasmodium white.

The plasmodia are usually large, and they often leave the wood to form the sporangia on grass and dead leaves in the neighbourhood. It shows no very close resemblance to the typical *S. fusca*, for the sporangia are short, closely set, practically sessile, of a darker dusky brown or nearly black colour, and the hypothallus forms ridges round the base of the sporangia. As the peripheral net is lacking, it would perhaps be better to transfer it to *Comatricha*, as has been done with *Stem. splendens* var. *flaccida* (cf. *Comatricha flaccida*).

Stem. herbatica Peck

Among my specimens which answer the description of this species, two groups are represented; those of the first group are tall, over 1 cm high; these grow in tufts on dead wood; the other ones are shorter, not exceeding 0.7 mm, more densely tufted with the tufts standing close together; these are found on grass and dead leaves. In the specimens growing on wood the plasmodium is white or yellow, in the other ones always white.

Stem. hyperopta Meylan var. *hyperopta*

On dead coniferous wood; common. Plasmodium white.

This species is easily recognizable, because it is of a colour which is most unusual in this genus, viz. pale lilac-rosy; in time, however, the sporangia darken, although they always retain a trace of the original lilac colour. The spores have a very characteristic but faint reticulation, with a small mesh where the spores touch each other in the sporangium, and larger, often elongate meshes in between.

As the surface net is present only in the basal part of the sporangium, and as it is even here often incomplete, this species might be placed equally well with *Comatricha*, a fact which in the beginning caused me a good deal of trouble. My first collections were identified by Dr. Karstens.

* *Stem. hyperopta* Meylan var. *microspora* Lister

Two collections from wood; these are much like the typical form; one from dead leaves and living grass; here the sporangia are more conical than in the typical form.

Stem. pallida Wingate ?

With some hesitation I place here one sporangium from a large beech stump, and a small collection from an oak branch.

Stem. splendens Rost. var. *flaccida* Lister cf. *Comatricha flaccida*

* *Stem. smithii* Macbride

On dead wood; common. Plasmodium white.

Much like *S. axifera*, but differing in the cinnamon-brown colour of the sporangium and the smaller size of the spores.

Stem. trechispora (Torrend) Macbride ?

Here I place, although perhaps erroneously, some sporangia found in tufts on leaves; the spores are beautifully banded-reticulate; further study is necessary. Plasmodium white.

* *Stem.* spec. ?

A very dark, almost black Myxomycete, twice collected. The sporangia are closely appressed, more or less like those of *S. fusca* var. *confluens*, with little or no surface net; the columellas weak and crooked or wanting; the capillitium wide-meshed, very dark; the spores very conspicuously marked with a broken, banded reticulation. Parts of the peridium prove to be persistent, and show faint dashlike marks. Dr. Martin suggests that it may be an *Amaurochaete*, but the presence of peridium parts between the sporangia seems to contradict this. It might perhaps find a place in *Comatricha*, in the vicinity of *C. flaccida*. I hope to return to this species at another occasion, when it has been studied more fully.

Trichia botrytis (J. F. Gmel.) Pers. var. *botrytis*

In autumn and winter on decaying wood; common. Plasmodium black.

* *Trich. botrytis* (J. F. Gmel.) Pers. var. *munda* Lister

Once collected on a leaf. Plasmodium brown.

The collection consists of a very small number of sporangia; the latter are minute and brownish-yellow.

* *Trich. botrytis* (J. F. Gmel.) Pers. var. *flavicoma* Lister

Once found, in a culture on bark taken from a living willow at Schaloen, South Limburg. Plasmodium white.

Trich. contorta (Ditmar) Rost. (in this species I include *T. inconspicua* Rost.)

On bark and wood of dead trees; not rare. Plasmodium yellow-brown.

It seems to me that *T. contorta*, at least as interpreted by Lister, is no more than an abnormally developed *T. inconspicua*. I am inclined to enclude in this species also *Hemitrichia karstenii*, as in the latter plasmodiocarpous forms often occur side by side with sporangiate ones, and as in the plasmodiocarpous forms the anastomising elaters are, as a rule, neither very conspicuous nor numerous, at least in the specimens I have doubtfully refered there.

Trich. decipiens Macbride cf. *T. pusilla*

Trich. favoginea (Batsch) Pers.
Four collections from tree stumps. Plasmodium yellow.
Mrs M. L. Farr (Mycologia **50**: 357–369. 1958) has united *T. persimilis*, *T. affinis* and *T. favoginea* to one species, as in her opinion, based on a study of a large number of specimens, they merge one into the other. However, as there are in my material no transitions at all between *T. persimilis* and *T. flavoginea*, I have kept them apart.

* *Trich. floriformis* (Schw.) G. Lister
One collection from a beech stump in the park "Hoge Veluwe".

Trich. persimilis Karst.
On dead wood; common. Plasmodium white.
See the remark made under *T. favoginea.*

Trich. pusilla (Hedw.) G. W. Martin (*T. decipiens* Macbride)
On decaying wood; common. Plasmodium white or pink.

Trich. scabra Rost.
On decaying wood and sometimes on straw; very common. Plasmodium white.

Trich. varia (Pers.) Rost.
On dead wood and bark; very common; more rarely on leaves. Plasmodium white.

Trich. verrucosa Berk.
A few collections only, among others in several consecutive years from a hole in an oak stump. Plasmodium white.

Tubifera ferruginosa (Batsch) F. J. Gmel.
On decaying wood; very common. Plasmodium orange or salmon-coloured.
In the sporangia composing the pseudoaethalium I often find pseudocapillitium, rigid tubes or (and) pseudocolumellae.

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TRANSLOCATION OF 3-AMINO-1, 2, 4-TRIAZOLE IN PLANTS

II. INHIBITION STUDY

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(received December 10th, 1960)

Introduction

In a previous paper (Massini, 1958) the uptake and translocation of 3-amino-1, 2, 4-triazole (AT) and 3-hydroxy-1, 2, 4-triazole (OT) had been studied. It was concluded that AT was translocated by both phloem and xylem vessels, whereas OT was only mobile within the xylem system. The present paper reports a study of the translocation and its inhibition by light-starvation and by cyanide of amino-triazole.

Materials and methods

Seedlings of tomato and French dwarf bean have been used as described earlier (Massini, 1958). AT, labelled with C^{14} in the 5-position with a specific activity of 2 mc/mmole was used. For foliar uptake, 10 μl of a 0.05 M solution of AT was applied to one leaf either by smearing it on within a ring of lanoline that had a diameter of 12 mm, or by the leaf injection method of Biddulph (1941). For uptake through the stem, the roots of bean plants were cut off under water and the plants placed in Erlenmeyers flasks containing a 5.10^{-5} M solution of AT.

The translocation of AT was inhibited by keeping a section of the stem or of a petiole in an atmosphere containing HCN prior to and during the treatment with AT. Small cylindrical gas chambers were constructed from perspex. The chambers consisted of two halves of a hollow cylinder with a length of 30 mm and a diameter of 16 mm (internal dimensions). One half-cylinder was lined with filter paper which was soaked with 0.1 cc of cyanide buffer (Robbie, 1948). The buffer contained 0.5 M KOH and KCN at a concentration such that a solution of physiological pH which was in equilibrium with the buffer via the gas phase acquired a certain constant concentration of HCN. The concentrations of KCN used and the corresponding equilibrium concentrations of HCN are listed in Table 1.

Table 1

Composition of gas chamber fluid according to Robbie (1948)

Concentration of KCN (M) in 0.5 M KOH	Equilibrium concentration of HCN at 20° C (M)
0.50	4.6×10^{-5}
2.0	1.8×10^{-4}
5.5	4.6×10^{-4}

A section of petiole or stem was enclosed between two half-cylinders and a gas-tight seal made with lanoline. The chamber was supported in an appropriate way, so that the stem was not loaded by its weight. Fig. 1 shows a gas chamber mounted on the petiole of the third leaf of a tomato plant. The lateral leaflets were cut off in order to expose a sufficient length of petiole. In this experiment AT was applied by the leaf smear method.

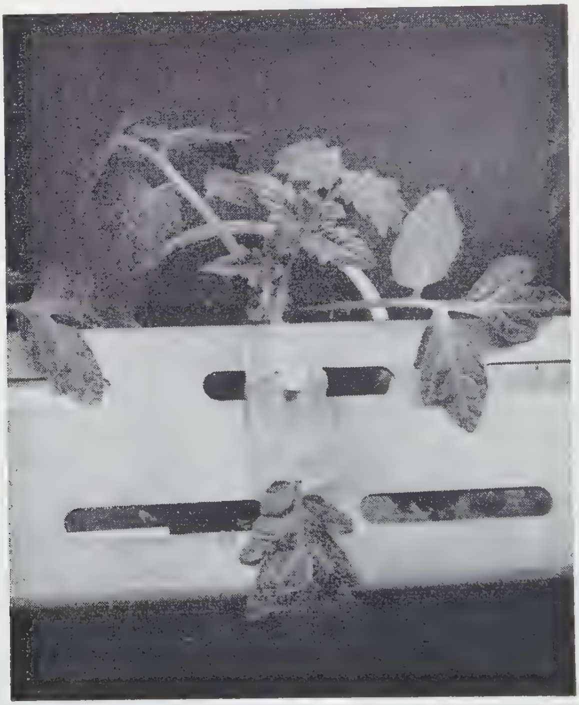

Fig. 1. Tomato plant with gas chamber mounted for inhibition experiment. AT was applied to the leaflet protruding from the gas chamber.

The control plants were treated in the same way except that the lining of the gas chambers was soaked with 0.5 M KOH.

The plants were mounted in a hood under a row of fluorescent lamps (light intensity 2000 $\mu W/cm^2$). After 5 hours of pretreatment with cyanide the solution of AT was applied, and after 17 to 19 hours of translocation the plants were harvested and cut into parts. These parts were extracted at room temperature, first with 10 cc of 70 % alcohol containing 5 % formic acid, then with 10 cc of water. Previous experiments had shown that with this procedure 80 to 90 % of the radio-activity was extracted. An aliquot of the collected extracts was evaporated on an aluminium planchet and assayed for radioactivity.

The influence of cyanide on the respiration was studied manometrically in a Warburg respirometer, at 20° C in the dark. Each

vessel contained: a disc of filter paper, soaked with 0.2 cc of water; some 600 mg of petiole sections (tomato) or 400 mg of stem sections (bean), cut to pieces of 5 to 10 mm; 0.6 cc cyanide buffer or 0.5 M KOH in the side-arm. The atmosphere was air. The measurement of the respiration was started 15 minutes after closing the vessels and continued for 3 hours.

For the starvation experiments bean plants grown in a gravel culture were used. The dark plants were kept in the dark for 48 hours, then treated with AT under a red safelight, then put back in the dark for another 24 hours. The light plants were grown in 16 hours light per day and kept in 24 hours of continuous light after the treatment with AT. 42 μg of AT was applied to a primary leaf, either by the leaf smear or by the leaf injection method. After the translocation period, the treated leaf was cut off and the treated area punched out and discarded. The treated leaf and the rest of the plant were extracted separately and the extracts assayed for radioactivity.

Results

The results of the inhibition experiments are compiled in Table 2. In experiment 1 the plants were mounted and treated as shown in Fig. 1. After the translocation period the treated leaf, including the petiole section enclosed in the gas chamber, was discarded and the rest of the plant assayed for radioactivity. The translocated amount is expressed in this experiment as a percentage of the amount applied. With 4.6×10^{-5} M HCN the inhibition was not significant, but 4.6×10^{-4} M HCN inhibited the translocation almost completely. The lower concentration inhibited the respiration of petioles by 50 %; with the higher concentration the inhibition was 75 %.

After one experiment the respiration of some non-treated leaves of a plant that had been treated with 4.6×10^{-4} M HCN (cf Fig. 1) was measured manometrically; it did not differ from the value for untreated plants. It seems thus that the action of the HCN is localized to the treated leaf.

In experiments 2 and 3 bean plants were treated in an analogous manner by applying AT to a primary leaf by the two methods indicated. The gas chamber was mounted around the stem above the node of the primary leaves, and after the translocation period the treated leaf (without the treated area in experiment 2), the top (above the chamber) and the rest of the plant were assayed separately. The translocation past the gas chamber is expressed as activity in the top in percent of the total activity in the plant. In both experiments 4.6×10^{-4} M HCN inhibited the translocation of AT almost completely. This concentration also inhibited the respiration of stem sections almost completely, whereas with 1.8×10^{-4} M HCN the respiration was almost normal, although the effect on the translocation of AT was still very pronounced. The uptake of AT and the translocation to parts other than the top were not affected by the treatment with HCN.

TABLE 2

HCN inhibition of translocation

Exp. no.	Plant	Place of application of AT	Method of application	Place of gas chamber	Part assayed for translocation	Concentration of HCN, M	Translocation past chamber, % of amount taken up	Inhibition %	Ns. of plants
1	tomato	terminal leaflet 3rd leaf	leaf smear	Petiole 3rd leaf	whole plant except treated leaf	0	2.5	—	4
						4.6×10^{-4}	0.055	98	4
						0	3.7	—	4
						4.6×10^{-5}	3.2	14	4
2	bean	primary leaf	leaf smear	internode above primary leaf	top	0	13	—	2
						1.8×10^{-4}	6	50	3
						4.6×10^{-4}	1	92	3
3	bean	primary leaf	leaf injection	internode above primary leaf	top	0	3.0	—	3
						1.8×10^{-4}	0.21	93	3
						4.6×10^{-4}	0.22	93	3
4	bean	through stem		internode above primary leaf	top	0	46	—	3
						1.8×10^{-4}	43	—	3
						4.6×10^{-4}	57	—	3

In experiment 4 the AT was fed through the stem. The gas chambers were mounted on the same place and the plants were assayed in the same manner as in the other experiments. In this experiment the translocation past the treated section was not inhibited by 4.6×10^{-4} M HCN (the apparent slight stimulation was not significant).

After the translocation period the treated stems had often lost their turgescence or were even shrivelled completely. The inhibition of translocation was not reversible; after a treatment sufficient to produce inhibition the stems did not recover again.

The results of the starvation experiments are listed in Table 3. It is shown that the translocation out of the treated leaf is strongly dependent on light. In the leaf smear experiment the darkened plants absorbed more AT than the light plants, possibly because in the former the treated area stayed wet for a longer time due to the lower surface temperature.

TABLE 3

Influence of light on uptake and translocation of AT by bean plants. (2 plants per treatment)

Method	Treatment	μg of AT taken up [1]	Translocated [2]	Translocation %
Leaf smear	dark	4.6	0.03	0.7
,, ,, ...	light	2.4	0.5	23
Leaf injection	dark	8.2	0.04	0.5
,, ,, ...	light	8.8	1.0	11

1) Without treated area.
2) Out of the treated leaf.

Attempts were made to replace the light by sucrose: starved bean plants were painted with a 0.3 M solution of sucrose two hours and one hour before the treatment with AT. However, the translocation out of the treated leaves was the same as in the starved control plants.

DISCUSSION

Dependence of translocation in plants on photosynthesis or some other source of carbohydrates had been found earlier. Several authors found that the translocation of (2,4-dichloro-phenoxy) acetic acid was stimulated by light or by application of sucrose (MITCHELL and BROWN, 1946; ROHRBAUGH and RICE, 1949; and others). HAY and THIMANN (1956) found the same dependence for the translocation of phosphate. In our experiments the light could not be replaced by sucrose, but this failure may be caused by an experimental difficulty rather than by a fundamental difference.

The inhibition of the translocation of phosphate by respiratory poisons has been described by KENDALL (1955). WILLENBRINK (1957)

made a thorough investigation on the inhibition of phloem transport of various inorganic and organic substances. He found that the translocation from a leaf of Pelargonium zonale of C^{14} labelled assimilates, of fluorescein, of N and of P compounds could be inhibited reversibly by incubating the phloem tissue of the petiole in an atmosphere containing HCN. The atmosphere was in equilibrium with a 10^{-3} M solution of KCN. It is difficult to compare the concentrations of HCN, on the one hand because Willenbrink's KCN solution was not buffered, and on the other hand because his technique—exposure of the central phloem tissue directly by cutting away the surrounding tissue—greatly facilitated the action of the poison.

In our experiments the action of the HCN was not reversible, and the wilting of the treated stems suggests that the poison may have killed the tissue. So it is not possible to decide if inhibition of the respiration alone is sufficient for a blocking of the translocation. The concentration of HCN which blocked the translocation also inhibited the respiration of the stem sections, but the degree of inhibition of both processes was different at different concentrations.

Comparison of experiments 3 and 4 indicates that in the bean plant the translocation of AT from a leaf to the top requires the presence of living tissue, and presumably occurs in the sieve tubes, whereas the same substance taken up by the transpiration stream passes through xylem tubes by a mechanism which is independent of living tissue. In this respect the conclusions arrived at in the previous paper and which were based on the distribution of AT in the plant after application to different parts, could be confirmed by the experiments described here.

SUMMARY

The translocation of AT out of leaves of tomato and bean plants has been found to be dependent on light and to be inhibited by HCN. The transport of AT taken up by the transpiration stream was not inhibited by the poison. The concentration of HCN which blocked translocation also inhibited the respiration of stem sections.

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THREE NEW REDUCTIONS IN THE ANACARDIACEAE

M. JACOBS

(Foundation Flora Malesiana, Leyden)

(*received October, 18th, 1960*)

Pentaspadon

A few botanists seem to have had difficulties in placing species of *Anacardiaceae* under the proper genus, even after Hooker gave an excellent key to 21 genera in the Fl. Br. Ind. **2**: 7. 1876. When Mr F. H. Hildebrand, engaged in identification of Papuan trees at the Rijksherbarium, Leyden, expressed his doubts of the generic identity of *Rhus novo-guineensis* Laut., and suggested that it might be a *Pentaspadon*, an attempt was made to verify this idea.

Lauterbach described *Rhus novo-guineensis* in 1910. Both specimens mentioned by him, *Versteeg 1314* and *1802*, are in the Rijksherbarium, and on examination both appeared to belong undoubtedly to *Pentaspadon*. The genera *Rhus* and *Pentaspadon* differ in the styles, 3 in number in *Rhus*, 1 in *Pentaspadon;* furthermore in *Rhus* the stamens are all equal, though sometimes reduced in ♀ flowers, whereas in *Pentaspadon* 5 stamens alternate with 5 staminodes. The fruit in *Rhus* is small, like a currant, in *Pentaspadon* it has the size of an almond.

The genus *Pentaspadon* was described first by Hooker in 1860, with *P. motleyi* from Borneo as only species. Shortly afterwards this species was described from Sumatra as *Nothoprotium sumatranum* Miquel. In his Flora of British India **2**: 28. 1876, Hooker described (with doubt as to the generic identity) *P. velutinus*, the second species in the genus. Engler's transfer of this species to *Microstemon*, a new monotypic genus established by him in Bot. Jahrb. **1**: 376. 1880, was merely founded on a wrong observation, as Corner rightly pointed out in Gard. Bull. S.S. **10**: 261. 1939.

The main differences between *P. motleyi* Hook.f. and *P. velutinus* Hook.f. consist of the glabrous appearance and lax inflorescence of the former, whereas the latter is densely velvety fulvous-hairy on all parts and its inflorescence is dense.

It is to *P. motleyi* Hook.f. that *Rhus novo-guineensis* Laut. has to be reduced. The species is widely distributed in Sumatra, Borneo, Ceram, and New Guinea. *Pentaspadon velutinus* occurs in central and northern Malaya and in eastern Sumatra.

Another species described by Lauterbach in 1920 is *P. moszkowskii*. The type specimen, *Moszkowski 283*, was probably destroyed with the Berlin Herbarium. From Lauterbach's description and the fine drawing he gives, it is justified, I think, to reduce also this species to *Pentaspadon motleyi*, the variability of which well covers the characters given for *P. moszkowskii*.

The essential synonymy is as follows:

Pentaspadon motleyi Hook.f., Trans. Linn. Soc. **24**: 168, t. 24. 1860. — *Nothoprotium sumatranum* Miq., Sumatra 527. 1862. — *Rhus novo-guineensis* Laut., Nova Guinea **8**: 298. 1910; Bot. Jahrb. **56**: 363. 1920. — *P. moszkowskii* Laut., Bot. Jahrb. **56**: 358, f. 2. 1920.

It is possible that more reductions in *Pentaspadon* must follow, when type material is brought together. According to CORNER, *P. officinalis* Holmes ex King and *P. velutinus* are exceedingly similar. The generic identity of *P. curtisii* (King) Corner is, according to CORNER himself, not quite certain. The description of *P. minutiflora* Burtt, Kew Bull. 305. 1935, from the Solomons, tallies well with that of *P. motleyi*. The next paragraph deals with the reduction of *P. teleianthera* Ridl.

SOLENOCARPUS

The examination of *Pentaspadon teleianthera* Ridl. showed that it belongs to *Solenocarpus*. *Pentaspadon* and *Solenocarpus* are closely related genera. Both of them have pinnate leaves, paniculate inflorescences, bisexual 5-merous flowers, while the pistil consists of 1 carpel with an oblique style and stigma, oddly shaped. *Solenocarpus* has 10 fertile stamens and a long clavate style with elongate stigma and a fruit ± ¾ cm long, whereas *Pentaspadon* has 5 fertile stamens and 5 staminodes, a short style with bilobed stigma, and a fruit of ± 2½–3 cm long.

Solenocarpus was described with one species, *S. indica*, by WIGHT & ARNOTT, Prod. 171. 1834; it occurs in the SW. part of the Indian Peninsula. The second species, *S. philippinensis* (Elm.) Kosterm., was transferred to it from *Pegia* by KOSTERMANS in 1955, who at the same time incorporated in this species the monotypic *Skoliostigma defolians* Laut. from New Guinea. Judging from the plate that LAUTERBACH gave, this is undoubtedly correct.

The type specimen of *Pentaspadon teleianthera* Ridl. from Borneo, Sarawak, *Haviland 2871*, has only very young leaves, while it is in full flower. From this and other material it can be concluded that the plant is deciduous, and bare when flowering. According to KOSTERMANS it is distributed in Sumatra, Borneo, the Philippines, Celebes, and New Guinea. The essential synonymy is as follows:

Solenocarpus philippinensis (Elm.) Kosterm., New and Crit. Malays. Pl. **3**: 1. 1955. — *Pegia philippinensis* Elm., Leafl. Philip. Bot. **8**: 3100. 1919. — *Skoliostigma defolians* Laut., Bot. Jahrb. **56**: 356, f. 2. 1920. — *Phlebochiton philippinensis* (Elm.) Merr., En. Philip. **2**: 472. 1923. — *Pentaspadon teleianthera* Ridl., Kew Bull. 199. 1933.

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BRIEF REPORTS

GADELLA, T. W. J.: The chromosome number of Anthocleista djalonensis Chev.

Few cytological data are available of the *Loganiaceae*. Its subfamily *Buddleioideae*, often considered a separate family, is a well-defined group, as far as could be concluded from the chromosome number. On the other hand, nothing can be said with certainty of the other subfamily, the *Loganioideae*, because the available data are still insufficient. Hitherto, the chromosome numbers of the following seven species of *Loganioideae*, studied by MOHRBUTTER (1936) and MOORE (1947), are known:

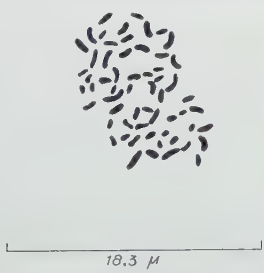
Gelsemium sempervirens	2n = 16	(MOORE, 1947)
Strychnos laurina	2n = 24	(MOHRBUTTER, 1936)
Strychnos nux-vomica	2n = 24	(MOHRBUTTER, 1936)
Strychnos sansibariensis	2n = 24	(MOHRBUTTER, 1936)
Spigelia marilandica	2n = 48	(MOORE, 1947)
Fagraea fragrans	2n = 12	(MOHRBUTTER, 1936)
Fagraea litoralis	2n = 12	(MOHRBUTTER, 1936)

These data seem to indicate that the basic chromosome number of the *Loganioideae* is X = 6.

The chromosome number of none of the species of the genus *Anthocleista* being known, Dr. Leeuwenberg asked me to examine *Anthocleista djalonensis* Chev., of which he collected herbarium material (Leeuwenberg 3285, WAG, UC) and mature fruits near Bouaké in the Ivory Coast in 1959. The chromosome number of this species might give valuable indications with regard to the relationship of this genus with the other genera of *Loganiaceae*.

Seedlings of this herbarium number have been grown in the greenhouses of the Hortus Botanicus at Utrecht and of the State Agricultural University at Wageningen.

Roottips were fixed in Karpechenko, embedded in paraffin, sectioned at 15 μ, and stained according to Heidenhain's haematoxylin method. The drawing was made with the aid of a "Carl Zeiss-Zeichenaufsatz".



Anthocleista djalonensis Chev.
2n = 60

The chromosomes are very small, rod-shaped, 0,7–1,5 μ long, i.e. they have about the same size as those of *Spigelia marilandica*. The number, $2n = 60$, is in accordance with other numbers known in the *Loganioideae* up to the present.

If the basic number for *Anthocleista* should, indeed, be $X = 6$, *Anthocleista djalonensis* would be dekaploid. However, further cytotaxonomic investigations of this genus and of other genera of *Loganioideae* are needed to corroborate this statement.

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BOOK REVIEWS

OF PUBLICATIONS RELATED TO BOTANICAL WORK IN THE NETHERLANDS

H. Lundegårdh, Pflanzenphysiologie, VEB Gustav Fischer Verlag, Jena, 1960. DM. 48.70. XVI + 717 blz., 283 fig.

Dit boek is een — tot 1958 bijgewerkte — Duitse uitgave van het uit 1950 daterende Zweedse leerboek van de schrijver; blijkens de inleiding is het bedoeld zowel voor gevorderde studenten als voor jonge onderzoekers. Er mag dus een zekere hoeveelheid voorkennis bij de lezer aanwezig worden geacht en zo is een grote moeilijkheid opgelost die zich bij het schrijven van een leerboek van de fysiologie voor beginners voordoet, dat men haast geen onderwerp behandelen kan zonder telkens vooruit te moeten grijpen op zaken die pas in latere hoofdstukken aan de orde komen. De zo verkregen grotere vrijheid in de behandeling van de stof blijkt op vaak aantrekkelijke wijze uit de indeling van het boek.

In het eerste hoofdstuk wordt over de cel en het protoplasma gehandeld en onder dat hoofd vinden we verenigd een behandeling van de microscopische structuur en van de structuurchemie van het cytoplasma, mitose (met niet zeer duidelijke figuren), meiose (zonder enige illustratie), bouw van de plastiden, chemie van de kleurstoffen, permeabiliteit, waterbalans van de cel, protoplasmastroming, plasmodesmen en celinsluitsels. Merkwaardig is dat de eerste illustratie de bouw van het electronenmicroscoop laat zien, terwijl de met dat instrument verkregen resultaten buiten beschouwing blijven. Hoofdstuk 2 geeft een vooral morfologisch getint overzicht van groei en ontwikkeling van de cel, terwijl de inleiding voltooid wordt met hoofdstuk 3, waar de anatomische grondslagen van fysiologische processen worden besproken. Al worden de lagere planten niet vergeten, de hogere staan steeds sterk op de voorgrond.

Achtereenvolgens worden nu behandeld de fotosynthese (met de chemie van koolhydraten en vetten), ademhaling en gisting, stikstof-stofwisseling (met de synthese van eiwitten en andere stikstof-houdende verbindingen, en de heterotrofie), opneming en transport van stoffen, waterbalans, groei, bewegingsverschijnselen.

Op verschillende van deze gebieden heeft de schrijver originele bijdragen geleverd en het verbaast dus niet, in vele hoofdstukken de opvattingen terug te vinden waartoe het eigen onderzoek de schrijver heeft geleid, evenwel zonder dat hiermee het werk van anderen verwaarloosd is. Het boek geeft zeer veel, veel ook dat men in de meeste boeken van deze omvang niet of niet zo aantreft en als zodanig is het zeer waardevol.

Naast veel reden tot dankbaarheid tegenover de schrijver voor wat hij ons geeft, is er op enige punten toch ook aanleiding tot kritiek. Zo wordt wel overal gewezen op veel — ook modern — onderzoek (de literatuurlijst achterin bevat ruim 2200 nummers), maar de resultaten van dat werk zijn niet steeds volledig in de tekst verwerkt. Het zojuist genoemde ontbreken van de resultaten van het onderzoek met het electronenmicroscoop is er een voorbeeld van; op p. 551 wordt vermeld dat bij de lichtgroeireacties van *Phycomyces* de latentietijd met het stijgen van de lichtintensiteit wordt verkort, en er wordt dan verwezen naar onderzoek

van CASTLE en HONEYMAN, zonder dat erbij wordt gezegd dat deze auteurs bij stijgende lichtintensiteit ook grotere hoeveelheden licht gaven; die verkorting van de latentietijd berust dan ook grotendeels op het eerder zichtbaar worden van een sterkere reactie. Op p. 534–535 wordt een onderzoek van ANKER met wortels vermeld, zonder dat de resultaten daarvan behandeld worden, en dat is heel gelukkig, want ANKER onderzocht de groei van coleoptielen, zonder er één wortel bij te gebruiken.

De enzymen zijn behandeld in het hoofdstuk „Atmung und Gärung". De ervaring leert dat die bespreking dan bij studenten de indruk kan wekken, dat enzymen vooral bij de ademhaling een rol spelen; hier lijkt nu de auteur zelf het slachtoffer te zijn van de plaats waar hij deze stof behandelt, wanneer hij op p. 229 mededeelt dat het enzymmolecuul uit apo- en co-enzym is opgebouwd. Dergelijk generaliseren vinden we meer; zo staat op p. 239: „Ein transportables Coenzym wird Hormon genannt" en even verder: „Weit verbreitete Hormone sind die Auxine, ..."; op p. 240: „Prosthetische Gruppen, die von einem Organismus zu einem anderen übertragen werden, sind die Vitamine".

Uit de verdere tekst blijkt vaak wel dat het niet zo kwaad gemeend is, maar er is hier toch sprake van een zekere slordigheid van uitdrukking, die zich bv. ook uit in de mededeling op p. 272, dat het wezen van bepaalde oxydaties is: „eine in kleinen Schritten fortlaufende *Verbrennung des Wasserstoffs* organischer Verbindungen" en op tal van andere plaatsen.

De figuren zijn in het algemeen goed, er zijn enkele uitzonderingen, waarvan fig. 4 op p. 4 wel het ergste voorbeeld is, daar men hier slechts met moeite ontwaart wat de bedoeling is geweest. In fig. 153 is een goede foto gegeven van een apparaat waar men weinig meer aan ziet, dan dat het nogal ingewikkeld is en dat er veel draden aan zitten.

Het schrijven van een leerboek van de plantenfysiologie door één persoon is een moedige onderneming geweest. De voordelen van grotere eenheid van behandeling, dan mogelijk is in een werk, waar velen aan hebben meegewerkt, springen het gehele boek door in het oog. Men moet dan evenwel de nadelen op de koop toenemen; van die nadelen is boven iets aangeduid. Zou het niet mogelijk zijn deze bij herdruk te omzeilen, zonder ook de voordelen te verspelen, door specialisten te vragen de verschillende hoofdstukken eens kritisch door te lezen?

H. P. BOTTELIER

PFLANZENSOZIOLOGIE. Eine Reihe vegetationskundlicher Gebietsmonografien. Herausgegeben von der Bundesanstalt für Naturschutz und Landschaftspflege und der Deutschen Akademie der Landwirtschaften, Institut für Landesforschung und Naturschutz. Band II.

Die zwergstrauchreichen azidiphilen Pflanzengesellschaften Mittel-deutschlands. Von Dr. rer. nat. habil. RUDOLF SCHUBERT, Dozent am Institut für systematische Botanik und Pflanzengeographie der Martin-Luther-Universität Halle. 32 figures, 28 plates and 6 maps. VIII, 235 pages. 1960. Half cloth 54,40 DM.

With this publication the author has considerably advanced the study of the European dwarf-shrub vegetations. It gives a detailed description and a critical discussion of the acidophilous communities rich in dwarf shrubs occurring in

Central Germany as well as a survey of the higher units of this vegetation type as they are found all over Europe and North Africa.

In the first chapter the author expounds his intention. He wishes to define and classify the *Calluna*-rich heath vegetations of Central Germany; to analyse the climatic, geomorphological and edaphic conditions found in the various *Calluna*-rich heath associations; to compare them with related associations found in other geographical regions; and finally to draft a system of acidophilous heaths, tundras and garrigues that are rich in dwarf shrubs.

His method of classifying the heathland communities is based upon the principle of the greatest possible floristic similarity and requires therefore a consideration of the total stock of species that are represented in an association; this makes it possible to recognize the characteristic combination of species. Under the latter is understood the group of species which occur equally in all sub-units. As characteristic species in the sense of Braun-Blanquet are absent in the heathland commuties the author was obliged to apply the method mentioned above. The resulting classification is certainly a very natural one. This proves at the same time the serviceability of the method applied by the author. Communities recognized by this method normally hold the same rank as associations defined by means of characteristic species.

To the floristic and quantitative data on the vegetation are added careful observations on environmental factors like soil, climate, historical (anthropogenic) influences, and on genetic and geographical aspects. Correlations are obviously present, which proves the value of the classification.

The first chapter ends with a survey of the geographical situation of Central Germany, its geomorphology, climate, soil types and of the distribution of the heath associations.

In the second chapter the associations are described; floristic, ecological and genetic details are given, and the associations are compared with corresponding dwarf-shrub communities in other parts of Central Europe. Association tables are given in the form of collective tables; the latter have the advantage that they are easily readable and offer a clear survey of the various units and their relations. The tables are accompanied by detailed descriptions of soil profiles.

Both dwarf-shrub-rich woodlands and treeless dwarf-shrubvegetations are taken into consideration. Natural *Calluna*-rich associations are described from exposed habitats and from regions above the tree-limit. The various associations are compared, ecologico-geographical groups of species are distinguished, and a survey is given on the German acidophilous dwarf-shrub and grassland vegetations.

In the third chapter a new classification is drafted of the acidophilous plant-communities found in Europe. Three classes are distinguished.

I. *Loiseleurio-Vaccinietea*, dwarf-shrub tundras on acid soil, characterized by the presence of arctic-alpine species, and occuring to the north or above the tree-limit; hence mostly natural communities. The class comprises five unions, two of which occur in arctic and three in alpine regions.

II. *Nardo-Callunetea*, Central European acidophilous heath vegetations, boreo-temperate, characterized by a high constancy of low boreomeridional Ericaceae and Genisteae. The class comprises three alliances, viz. 1. *Vaccinio-Genistetalia:* the Central and Northern European heath vegetations with dominant boreal and boreomeridional species, subdivided in four unions; in the latter the species composing

the respective characteristic combinations belong to different geographical elements (resp. boreal, boreomeridional, subatlantic, continental). 2. *Ulicetalia europaeae:* atlantic high heath vegetations of Western Europe, characterized by atlantic *Ulex*- and *Erica*-species particularly by *Erica cinerea;* they are subdivided in two unions with respectively mediterranean and boreal elements. 3. *Nardetalia strictae,* not discussed. The *Erica tetralix*-associations which are found in Northwest Germany and in the Netherlands, are not mentioned by the author. Consequently their place in the system remains uncleared.

III. *Cisto-Lavanduletea,* mediterranean garrigues on acid soil. Seven unions, characterized by different plant-geographical elements.

In the last chapter a summary is given, and it is stated that the units recognized by the author show similarity with the vegetation belts described by Schmid. A series of photographs gives an impression of the physiognomy of the described associations. Maps show the distribution of the heath associations in Central Germany, and also of the various classes, alliances and unions found all over Europe and in North Africa.

J. Th. de Smidt

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THE INFLUENCE OF MERISTEMATIC TISSUE AND INJURIES ON THE TRANSPORT OF TOBACCO MOSAIC VIRUS IN NICOTIANA TABACUM L. CULTIVAR. SAMSUN

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CONTENTS

1. INTRODUCTION

It has been stated by several authors that the tops of the stem, the axillary buds, and the roottips of a plant infected with virus are of influence on the rate and direction of virus-transportation. There has been, however, no agreement about the effect of these plant-parts. Some authors suggest an accelerating, others an inhibiting effect of the stemtop on the rate of transportation. This discrepancy can be explained by the difference in methods used by these authors, by the difference in hostplants and virus-material and by the limited number of experiments which were carried out. Moreover these experiments were founded on one principle, namely of removal of the parts, and the influence of the stemtops or the axillary buds was studied by comparing the rate of virus-transportation in normal plants with that in plants from which these parts had been removed (GRAINGER, 1933; BENNETT, 1940; ZECH, 1952).

This method has two distinct disadvantages. 1) Since removal of these plant-parts may eliminate more than one kind of tissue, it is impossible to determine which tissue had influenced the rate of virus-transport. The meristematic tissues such as the vegetation-point or the leaf-primordia, the young leaves, or parts of the stem may play a rôle. 2) Injuries are made during removal of these parts which may influence the metabolism of the plant and consequently the rate of virus-transportation. Neither the influence of wounding nor the influence of meristematic tissue on the rate of virus-transportation in a plant has ever been examined.

The purpose of our experiments was to verify the results reported in literature and to determine the effect of the influence of meristematic tissue on the rate and possibly the direction of virus-transport.

Plants of *Nicotiana tabacum* L. cultivar. Samsun were used for the experiments. The tobacco mosaic virus (*Nicotiana* virus 1 Smith) was chosen and will be indicated in this paper as TMV.

In spite of the difficulties mentioned above, a method of removal of meristematic parts and axillary buds was applied here. However, only very small stemtips [1]) of known morphology were removed and the influence of the wounding caused by this removal was studied.

When it became evident that wounding influenced the rate of virus-transportation positively, another method was sought by which the activity of meristematic tissue could be eliminated without wounding. Temporal inactivation of meristematic tissue was achieved by powdering the stemtip and the axillary buds with 2, 3, 5, 6-tetrachloro-nitrobenzene, Fusarex.

It was also desirable to study the influence of meristematic tissue in an active condition on the rate of virus-transportation. Transplantation of such a tissue into a plant cannot be performed without wounding, and the influences of other meristematic tissue or tops present

1) In this publication a stemtip is defined as the extreme point of the stem with a length of maximal 2 mm, mainly consisting of meristematic tissue. The term stemtop is used when it concerns parts of more than 2 mm.

in a whole plant are not excluded. To comply with all conditions a homogeneous tissue, callus-tissue of tobacco, cultivated in vitro, was chosen for a third group of experiments. It proved to be possible to introduce a prepared meristem of a stemtip of 0.2 mm or a young stemtip of 2 mm on the callus culture without wounding the callus and without loss of activity of the meristem. In this way the influence of either a meristem or a stemtip could be determined, excluding the other meristematic influences.

To determine the rate of virus-transportation in a plant it is necessary to detect which tissue is first reached by the virus-material after inoculation. If the top of the stem and the axillary buds or a part of these organs have an attractive action, the virus-material leaving an inoculated leaf will first pass via the stem to these extremities of the plant and then to the other leaves. The virus-transportation through the stem takes place at such a high rate that some parts of the stem may remain virus-free in spite of the fact that virus-material passed by (SAMUEL, 1934; KUNKEL, 1939; BEEMSTER, 1958). For this reason the stem is not suitable as an indicator of the rate of virus-transportation. Therefore the leaves were chosen to determine which places in a plant are first attacked by the virus-material. Parts of the leaves may also be passed by without retaining any infectious material. However, in the leaf as a whole, virus-material will be present.

Just after arrival in uninoculated leaves the amount of virus is too small to be determined with the existing methods and, moreover, it is possible that the virus-material is not yet infectious at the moment of arrival.

Presence of very small amounts of non-infectious virus-material in a leaf is demonstrable only after multiplication during a period of incubation. For this purpose the leaves were removed from inoculated plants. The leaves were placed with their bases into water and incubated for three days. Under this condition virus-material, already present in the leaves at the moment of cutting, was allowed to multiply during three days, after which infectious virus-material became demonstrable with the local lesion test on *Nicotiana glutinosa*-leaves. The presence of virus can be demonstrated also serologically but the local lesion test appeared to be the more sensitive of the two.

With this method, however, it is impossible to prove whether the meristematic tissue of the stemtip, the axillary buds or the leaf itself influences the virus-transportation. But if a stemtip or the axillary buds attract the virus-material, removal or inactivation of these plant-parts must change the rate of virus-transportation and the moment of appearance of virus-material in the adjacent leaves.

2. LITERATURE

2.1. VIRUS-TRANSPORTATION IN PLANTS

It is known that after inoculation of a leaf of a plant with a virus causing systemic infection, there is an initial period of virus-multiplication in local sites of the inoculated leaf. This period is followed by a

comparatively sudden appearance of virus in all parts of the stem, the roots and the topmost leaves. Later the virus spreads to the lower leaves (HOLMES, 1930; SAMUEL, 1934).

The way in which viruses are transported through a plant has already been described by many authors. A review of the literature is given by BEEMSTER (1958).

Several conflicting data concerning the way of virus-transportation are explicable by differences in test-objects, circumstances and place of inoculation. The latter seems to influence the direction of the first virus-transportation.

Many authors have experimented with tobacco plants and the tobacco mosaic virus. THUNG (1937) found that the higher the place of inoculation, the quicker the symptoms of virus will appear in the top. After inoculation of the lower leaves it is probably not the distance to the top that counts but possibly the age of the lower parts that causes a retardation of the reproduction of the virus and consequently a delay in transportation.

According to ZECH (1952) a rapid virus-transportation takes place from an inoculated leaf at the stembase down to the roots. After a long time, usually 15 to 30 days, the young top-leaves become infected. The mature leaves at the stem are infected very late or not at all. When the place of inoculation is in the region of the stemtop, the virus also moves directly downwards.

BENNETT (1940) inoculated one of the middle leaves of a tobacco plant with TMV and observed that virus-transportation took place both in apical and basipetal direction.

SAMUEL (1934) who inoculated a middle leaf of a tomato plant with TMV found virus-transportation down to the roots; shortly afterwards the virus moved from here to the top of the plant. When flowers or fruits were being formed, the virus was first transported in upward direction. Most authors agree that transportation occurs in a basipetal direction and that young shoots, flowers and fruits seem to attract virus.

Several authors have examined the influence of the removal of the stemtop or the axillary buds on virus-transportation. GRAINGER (1933) studied the influence exercised by the tops of the stems of tobacco plants. Leaves at the base of the stems were inoculated with TMV and, simultaneously, the top of the stem containing the vegetation-point, the leaf-primordia and the three youngest leaves, was cut from each plant. His results showed that the removal of the stemtop delayed the appearance of the virus-symptoms in the young leaves for two days and he concluded that the stemtop exercised an attractive action on the virus in the plant.

BENNETT (1940) found that 20 to 50 days were required for the virus to cause symptoms in the top of the plant after inoculation of the rootsystems. In a second series of experiments plants, approximately 15 cm high, were cut back after inoculation of the roots leaving a stem about five cm long from which, on most plants, lateral buds soon developed into shoots. This removal had a decided accelerating

influence on the appearance of symptoms in the new growth of the lateral buds. The symptoms appeared 6 to 8 days after the tops were removed. So, contrary to Grainger's opinion, Bennett concluded that the removal of the stemtop exercised an attractive action on the virus present in the roots. It is probable that the newly developed young shoots were attracting the virus. VALLEAU (1941) observed the same phenomenon in Burley tobacco, inoculated with TMV at topping time.

ZECH (1952) experimented with tobacco plants, 100 to 130 cm high. The plants were maintained in a vegetative state by breaking off the stemtop and the topmost laterals before inoculation with TMV. Twenty-six days after the inoculation of the middle or upper leaf a newly formed lateral had replaced the original stemtop. At that time the plants were tested for the presence of the virus. It was found, that the newly formed lateral was first to show virus-symptoms. Later on the virus moved into some leaves at the stem. When all the axillary buds were removed, from these plants all of the leaves became infected. However, this last experiment is not described in detail and the influence of the topping on the very first spreading of virus from the inoculated leaf was not determined. From his experiments Zech concludes that as long as the new stemtop is not invaded by the virus, it seems to have a remarkable, directing action on the spread of the infection. When the stemtop is infected, the direction is altered but from Zech's description it is not clear how this occurs. The stemtop can become infected after inoculation of the rootsystem only when the roots keep their vegetation-points. When the latter are cut, no upward virus-transportation takes place until new vegetation-points have developed. This experiment is not described in detail either. Zech also stated, that the developing of flower-buds or maturing fruits seems to surpass the influence of the roottops.

Objections may be raised against this kind of experiments. Removal of the roottops altered more than one factor. The growth of the plants was temporarily disturbed by removal out of the pots. Wounds are made by removing the tops of the roots, stemtops or axillary buds. The metabolism of the plant and therewith the multiplication and transportation of virus were influenced by the removal of vital parts as root- and stemtops and axillary buds. Also GRAINGER (1933) based his suggestion that the stemtip influences virus-transportation in plants on the results of his experiments in which stemtops were removed. Removal of stemtops, however, includes removal of the leaf-primordia and at least one young leaf because the vegetation-point is completely surrounded by these parts. It is technically impossible to remove the vegetation-point and leave the other parts untouched (Fig. 1, Plate 1 C). By removal of the axillary buds, embryonic tissue and primordia are cut out. Excision of these parts alters more than one factor and the plant as a whole is physiologically changed.

2.2. MERISTEMS

Meristematic tissue occurs in different places in a plant, and has been divided into two groups by FITTING (1947) according to the place

of occurrence and the nature of its origin. The *primary* meristems come into being during the division of the germ cell and build up the multicellular embryo. During post-embryonic growth and development primary meristematic tissue occurs mainly in the stem, roottips and in axillary buds. The *secondary* meristems originate from mature cells that are undergoing change of function and being transformed into embryonic cells by cell-division. Wound-meristems and cork-cambium may be considered as secondary meristems. According to KONINGSBERGER (1943) it is difficult to draw a line between primary and secondary meristems.

Several authors have investigated the structure of the stemtip. BUVAT (1955) distinguished the following three zones in the apical- or top-meristem. 1) The apex in which cell-division seldom occurs during the vegetative state; 2) a central zone, the medullar meristem, with differentiated cells, and 3) a lateral zone, the initial ring, with a strong meristematic character, in which most cell-divisions occur and from which leaf-primordia arise. The fine structure of meristematic cells and their components has been studied and described by WHALEY, MOLLENHAUER & LEECH (1960).

The origin, the structure and the functions of the apical meristem of a tobacco plant were studied extensively by FARDY, CUZIN & SCHWARTZ (1953). As long as the top-meristem is actively dividing, differentiation of the pro-cambial tissue lying underneath occurs. When the terminal meristematic region ceases dividing an axillary meristem becomes active.

2.3. DISTRIBUTION OF VIRUS IN TOBACCO PLANTS

Remarkably accurate observations concerning the division of virus in an infected plant were made by BEYERINCK as early as 1898. According to this author, only those organs of the plants with tissues that are in an active state of growth and in which cell-division takes place, are attacked by the virus. Mature tissue did not seem to be affected, but it allowed the transportation of the virus.

Quantitative determinations of the virus-distribution in the top of the stem have been made by LIMASSET & CORNUET (1949). They found a low virus-concentration in the outmost smallest top-leaves and a high concentration in the young, vigorously growing leaves underneath. A maximum concentration was frequently found in one of the leaves at the fifth to the tenth place, reckoned from the vegetation-point. No virus was found in the top-meristem of a tobacco plant inoculated with TMV.

Unpublished experiments by A. F. Schippers-Lammertse have shown that the top-meristem and frequently the first two leaf-primordia of a tobacco plant inoculated with TMV remain virus-free while the young leaves underneath contain virus in a high concentration and leaves in advanced stages in development contain less virus.

ZECH (1952) obtained the same results from his experiments: in the young leaves virus was present in a higher concentration than in

leaves farther from the top of the stem. In his experiments also the top-meristem itself was virus-free.

3. THE INFLUENCE OF REMOVAL OF MERISTEMATIC TISSUE ON THE RATE OF VIRUS-TRANSPORTATION

3.1. Material and methods

Plants of the *Nicotiana tabacum* L. cultivar. Samsun were used in all experiments. This variety was chosen because inoculation with TMV is always successful and results in systemic infections. It was also important to these experiments that leaves, cut from these plants retained their turgor for at least three days when their bases were placed in water. Moreover, this variety was suitable for the cultivation of callus-tissue. All plants were cultivated in the greenhouse and were used when they were six weeks old, in a six-leaf stage, with a height of 4 to 6 cm. If otherwise it will be mentioned in the individual experiments.

All experiments were carried out with a strain of *Nicotiana* virus 1 Smith, that shows distinct symptoms on Samsun tobacco plants. Sap, pressed out of leaves of tobacco plants that were infected with the strain of TMV and showed distinct virus-symptoms, was used as inoculation-material.

A leaf of a Samsun plant was dusted with carborundum-powder and then inoculated with sap containing TMV. After about one minute this leaf was rinsed carefully with water, taking care that the leaf did not touch other leaves and that the rinsing water did not reach the soil or other parts of the plant. The lowest, the middle or the topmost leaf was chosen as the place of inoculation.

The presence of TMV can be demonstrated easily by means of the test-plant *Nicotiana glutinosa* L., which shows local lesions when inoculated with TMV.

The following method was used to establish the arrival of virus-material in a leaf of a Samsun tobacco plant. All the leaves of an inoculated plant were cut off at specifical intervals after inoculation. After each cut the knife was disinfected. Each leaf was placed with its base in a test-tube containing some water. Under this condition, virus-material already present in the leaves was allowed to multiply for three days (Plate 1 A). After this period each leaf was pressed out and the sap was tested for the presence of virus. For this purpose a leaf of *Nicotiana glutinosa* was dusted with carborundum-powder and inoculated with the sap. After rinsing with water the leaves were placed in a petri-dish with moistened filter-paper under fluorescent light with an intensity of about 1000 lux. After 48 hours the number of local lesions on the leaves was counted. When more than two local lesions were present on one leaf it was assumed that the sap contained virus. Generally the number of local lesions per leaf varied from 8 to more than 100. The sap of the rootsystems was tested for the presence of virus too.

From a number of plants the stemtips or the axillary buds were removed simultaneously with the virus-inoculation. The stemtip, with a length of 2 mm, contained the top-meristem, the leaf-primordia and 3 to 4 very young leaves (Fig. 1, Plate 1 B). The axillary buds contained the top-meristem, the leaf-primordia and sometimes one to two very young leaves.

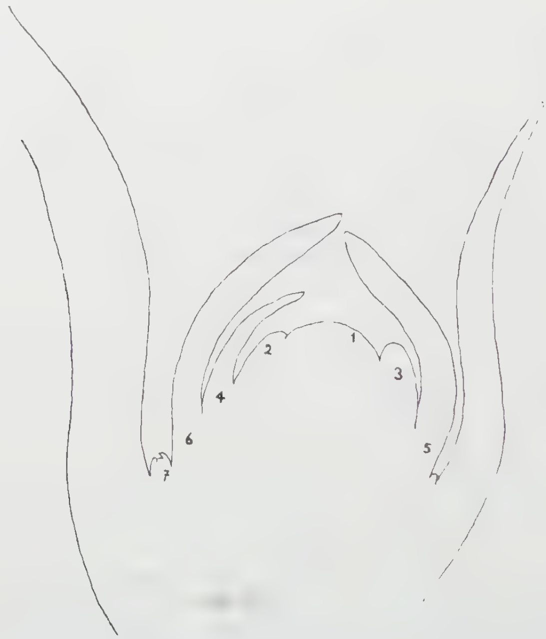

Fig. 1. Scheme of the stemtop of *Nicotiana tabacum* L. 1: youngest leaf-primordium. 2 and 3: older primordia with the beginning of the development of pro-vascular strands. 4, 5 and 6: young leaves in different stages of development. 7: axillary bud with leaf-primordia.

Wounding of a plant without removing tips or buds was achieved by gently scraping the epidermis of the stem either along the whole length at two opposite sides or in a circular three mm band circling the stem just under the stemtop. In this way only the cells of the epidermis were damaged or atmost the layer of parenchymatic cells just underneath, but not the more deeply located layers of cells.

3.2. Experiments on the influence of the place of inoculation on virus-transportation in the plant

To determine the influence of the place of inoculation on the rate of transportation of the virus, 18 plants in a four to six-leaf stage, six weeks old, were divided into six groups of three plants. Two groups were inoculated on the lowest leaf with sap containing TMV, two groups were inoculated on a middle leaf and two groups on the top-

most usable leaf. After 48 and 72 hours, respectively, the presence of virus in the leaves was determined by the local lesion method (Table I, exp. 1).

TABLE I

Influence of the place of inoculation on the rate of virus-transportation

Place of the inoculated leaf	Time after inoculation in hours	Virus-containing leaves		Ratio [3]		Virus-containing root-systems	
		exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2
lowest leaf [1]	48	1/11 [2]	2/13 [2]	0.08 ± 0.14	0.15 ± 0.11	0/3	0/3
,, ,,	72	4/12	6/12	0.33 ± 0.14	0.50 ± 0.00	0/3	1/3
middle leaf .	48	3/12	4/12	0.25 ± 0.00	0.33 ± 0.14	0/3	0/3
,, ,, .	72	6/13	8/12	0.47 ± 0.06	0.67 ± 0.14	0/3	0/3
topmost leaf.	48	1/14	1/11	0.07 ± 0.12	0.08 ± 0.14	0/3	0/3
,, ,, .	72	3/12	5/12	0.25 ± 0.00	0.42 ± 0.14	1/3	0/3

[1]) Three plants per group.

[2]) numerator: number of virus-containing leaves per group of plants excluding the inoculated ones; denominator: total number of leaves tested per group of plants excluding the inoculated ones.

[3]) Ratio of virus-containing leaves to ten tested leaves and standard-deviation. Second figure after decimal is approximate.

The number of virus-containing leaves of each group of tobacco plants is expressed in relation to the total number of leaves of the group tested. The inoculated leaves were not included, because they always contained virus. This ratio gives an indication of the spread of the virus through the plant. In a few cases only one leaf, and that at a large distance from the place of inoculation, contained virus. In such plants the ratio of virus-containing leaves is low, although the virus was transported a considerable distance. On the whole, however, the ratio of virus-containing leaves at a definite time appeared to be a good indication of the rate of virus-transportation.

Forty-eight hours after inoculation, of the blank number of leaves picked from plants inoculated on either the topmost or lowest leaf only one leaf contained virus. Of the blank number of leaves cut from the plants inoculated on a middle leaf, three leaves contained virus. Seventy-two hours after inoculation the number of virus-containing leaves was higher in all groups, the highest number was always reached in plants of which the middle leaf had been inoculated.

The fact that the number of virus-containing leaves per plant within one group can vary, makes it difficult to evaluate the differences stated between the groups. The standard-deviation of the ratio of virus-containing leaves of the plants within one group was calculated. In the experiments described in this paper, the differences between the groups were tested by Students "t"-test, as the number of testplants was rather small: three or five per group.

This experiment was repeated with six weeks old plants (Table I, exp. 2). In all groups the number of virus-containing leaves was somewhat higher than in the previous experiment. In this trial too, the inoculation of the middle leaf caused the highest rate of virus-transportation. From the two experiments it can be concluded that inoculation of a middle leaf of a plant causes the most rapid transportation of virus-material through the plant.

The fact that in practically all cases the rootsystems do not contain the virus, indicates that at least in our experiments the virus is not first transported to the roots before entering the leaves, as SAMUEL (1934) concluded.

3.3. Comparison of the rate of virus-transportation in plants with and without a stemtip

The next experiment was intended to determine the influence of removing the stemtip simultaneously with the inoculation of a leaf. Though inoculation of the middle leaf seemed to be most promising, in this experiment the lowest and the topmost leaf were also used as place of inoculation in order to compare the results of this experiment with those of the former.

a) *Inoculation of the lowest leaf*

Thirty tobacco plants were inoculated on the lowest leaf with sap containing TMV. Of half this number the stemtip of two mm was removed simultaneously with the inoculation. The removal took place with the aid of a very sharp knife, in order to make the injury as slight as possible. The 15 plants without the stemtip were divided into three groups of five plants of which the number of virus-containing leaves was determined 48, 96 and 120 hours, respectively, after inoculation. The plants with stemtips were treated in the same way. In Table II A a survey is given of the place of the virus-containing leaves.

It was not uncommon for a virus-free leaf or leaves to be found between two virus-containing leaves. There was no correlation between the position of the leaf and the time of appearance of the virus.

Forty-eight hours after inoculation, the ratios of virus-containing leaves of plants with and without stemtip were 0.14 and 0.13, respectively (Table II B). The virus had not yet penetrated into the rootsystems. Ninety-six hours after inoculation a ratio of 0.74 and 0.63, respectively, was found. Two of the five rootsystems of the plants with a stemtip were infected and all of those of the plants without stemtips. One hundred and twenty hours after inoculation a high number of the leaves contained virus in both groups while three of the five rootsystems of the plants with a stemtip were still virus-free.

From this experiment it appeared that the ratios of virus-containing leaves of plants with and without stemtip were not distinctly different. Removal of the stemtip simultaneously with the inoculation of the lowest leaf, has little or no influence on the rate of virus-transportation to the higher leaves in the plant. The only difference between the

TABLE II

The spreading of virus after removal of the stemtip of plants compared with that in plants with intact stemtips 48, 96 and 120 hours after inoculation of the lowest leaf.

A. Place of the virus-containing leaves at the stem of plants with intact stemtip.

hours after inoculation	48					96					120				
Plant number [1]) . .	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
6th leaf					–′	x′	–′						x′	x′	x′
5th ,, ...	–′	–′	–′	–′	–	x	x		x′	x′	x′	x′	x	x	x
4th ,, ...	–	x	–	x	x	x	x	x′	x	–	x	x	x	–	x
3rd ,, ...	–	–	–	–	–	–	x	x	–	x	–	x	x	–	–
2nd ,, ...	–	–	–	–	–	–	–	x	x	x	x	x	–	x	x
1st ,, ...	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
root-system	–	–	–	–	–	–	–	x	–	x	x	x	–	–	–

Place of the virus-containing leaves at the stem of plants without intact stemtip.

	48					96					120				
7th leaf		★		★	★										★
6th ,, ...	★	–		–	–	★		★	★	★	★				x
5th ,, ...	–	–	★	x	–	–	★	–	x	x	x	★		★	–
4th ,, ...	–	–	–	–	x	x	–	x	–	–	x	x	★	x	x
3rd ,, ...	–	–	–	–	–	–	x	x	x	x	–	x	x	x	x
2nd ,, ...	x	–	–	–	–	x	x	x	–	x	x	x	x	x	x
1st ,, ...	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
root-system	–	–	–	–	–	x	x	x	x	x	x	x	x	x	–

B. Calculation of the number of virus-containing leaves.

Presence of stemtip	Time after inoculation in hours	Virus-containing leaves	Ratio [3])	Virus-containing rootsystems
+	48	3/21 [2])	0.14 ± 0.13	0/5
+	96	15/21	0.74 ± 0.16	2/5
+	120	18/23	0.79 ± 0.14	2/5
—	48	3/22	0.13 ± 0.12	0/5
—	96	12/19	0.63 ± 0.13	5/5
—	120	15/17	0.91 ± 0.12	4/5

[1]) Five plants per group.
[2]) and [3]) See note Table I.
′ stemtip.
★ stemtip removed.
● inoculated leaf.
– leaf or rootsystem without virus.
x leaf or rootsystem containing virus.

plants with and without a stemtip seemed to be a somewhat faster virus-transportation to the rootsystems after removal of the stemtip. It is possible that the lowest leaf as a virus-source is too far away from the stemtip, to be influenced by its presence or absence.

b) *Inoculation of the middle or the topmost leaf*

Forty plants were divided into eight groups of five plants. Of the plants of four groups the stemtips were removed simultaneously with the inoculation. Two groups of plants with and two groups without the stemtip were inoculated on the middle leaf and the other four groups on the topmost leaf that still could be inoculated. After 48 and 72 hours it was determined in which leaves virus was present (Table III).

TABLE III

Rate of virus-transportation after inoculation of the middle leaf or the topmost leaf of plants with and without stemtip.

Place of the inoculated leaf	Presence of stemtip	Time after inoculation in hours	Virus-containing leaves	Ratio [3]	Virus-containing rootsystems
middle leaf [1]) . . .	+	48	6/19 [2])	0.31 ± 0.06	0/5
,, ,, ...	+	72	12/19	0.63 ± 0.13	3/5
,, ,, ...	—	48	5/18	0.28 ± 0.04	0/5
,, ,, ...	—	72	13/19	0.68 ± 0.11	2/5
topmost leaf	+	48	2/24	0.08 ± 0.11	0/5
,, ,, ...	+	72	10/24	0.42 ± 0.04	2/5
,, ,, ...	—	48	1/18	0.05 ± 0.11	0/5
,, ,, ...	—	72	8/19	0.42 ± 0.12	3/5

[1]) Five plants per group.
[2]) and [3]) See note Table I.

The number of virus-containing leaves in the plants still in possession of the stemtip does not differ distinctly from that of the plants without stemtip: 48 hours after inoculation of the middle leaf the ratios were 0.31 and 0.28, respectively; 72 hours after inoculation the ratios were found to be 0.63 and 0.68, respectively. After inoculation of the topmost leaf the rate of virus-transportation was much lower than after inoculation of the middle leaf: a ratio of only 0.08 of the leaves of the plants with a tip and a ratio of 0.05 of those without the tip was found when tested 48 hours after inoculation. When tested 72 hours after that treatment the ratio was 0.42 for both groups of plants. The virus had not penetrated any of the rootsystems during the first 48 hours after inoculation. The number of infected rootsystems at 72 hours after inoculation did not appear to be influenced by the removal of the stemtip or the place of inoculation.

From the two experiments the conclusion could be drawn that inoculation of the middle leaf caused the most rapid virus-transportation to the other leaves of the plants. Therefore in the next experiments the middle leaf was always chosen as the place of inoculation.

In the preceding experiments there was no distinct difference in the rate of spreading of virus-material through the plant between plants with and those without the stemtip, neither in the transport to the leaves nor to the roots. The removal of the stemtip of a plant does not seem to influence the rate of virus-transportation out of the inoculated leaf whatever its place. No attracting or directing action of the stemtip could be demonstrated by removing this part of the plant.

3.4. Experiments on the influence of the removal of the axillary buds on the rate of virus-transportation

Although removal of the stemtip simultaneously with the inoculation does not influence the rate of spreading of the virus through the plant, it is possible that removing all the axillary buds does have an influence. To answer this question, the middle leaves of 24 plants were inoculated. The plants were divided into 4 groups of 6 plants. From the plants of the first group no axillary buds or stemtips were removed; from those of the second group the stemtips were cut out;

Table IV

Influence of removal of the stemtip and/or the axillary buds of plants on the rate of virus-transportation.

Experiment number	Group number [1]	Removal of	Time after inoculation in hours	Virus-containing leaves	Ratio [3]	Virus-containing root-systems
1	1	—	72	2/13 [2]	0.15 ± 0.13	0/3
			96	4/14	0.28 ± 0.10	0/3
	2	stemtips	72	2/11	0.17 ± 0.14	0/3
			96	4/12	0.33 ± 0.14	0/3
	3	axillary buds	72	5/12	0.42 ± 0.14	0/3
			96	8/12	0.67 ± 0.14	0/3
	4	tips and buds	72	5/10	0.50 ± 0.17	0/3
			96	8/12	0.67 ± 0.14	0/3
2	5	—	48	1/11	0.08 ± 0.14	0/3
			72	4/11	0.36 ± 0.12	0/3
	6	axillary buds	48	4/12	0.33 ± 0.14	0/3
			72	6/12	0.50 ± 0.00	0/3
3	7	—	72	2/12	0.17 ± 0.14	0/3
			96	4/14	0.28 ± 0.10	0/3
	8	axillary buds	72	6/13	0.47 ± 0.06	0/3
			96	10/13	0.77 ± 0.03	1/3

1) Three plants per group.
2) and 3) See note Table I.

from those of the third group all the axillary buds; and from the plants of the fourth group both stemtips and axillary buds were taken away. All of these treatments were performed simultaneously with the inoculation.

From each group the presence of virus in the leaves and the rootsystems was tested 72 and 96 hours after inoculation (Table IV, exp. 1).

The virus had not penetrated the rootsystems. Seventy-two hours after inoculation the spreading of the virus in the plants of the first group with a ratio of virus-containing leaves of 0.15 did not differ distinctly from that of the second group with a ratio of 0.17. In the third and fourth groups the rate of virus-transportation was higher showing a ratio of 0.42 and 0.50, respectively. Ninety-six hours after inoculation the same picture occurred: removal of the stemtip did not influence the virus-transportation, removal of the axillary buds did. In all groups the virus went upwards and downwards. It could be concluded that removal of the axillary buds caused a higher rate of virus-transportation from the inoculated leaf to other leaves.

Two more experiments were carried out to investigate the influence of removal of all the axillary buds (Table IV, exp. 2 and 3). It was again shown that removal of the axillary buds caused a higher rate of virus-transportation.

It can be asked, however, which factors are of influence on the rate of virus-transportation. It is possible that the loss of meristematic tissues by removal of all the axillary buds exercises an effect, but it also may be that the effect is due to the wounds necessarily made, followed by wound-reactions. Perhaps a combination of these factors is responsible for the increased rate of virus-transportation.

4. INFLUENCE OF WOUNDING ON THE RATE OF VIRUS-TRANSPORTATION IN TOBACCO PLANTS

4.1. Literature

Each injury, inflicted upon a living plant, causes changes in the damaged cells or tissues. These changes can be confined to the immediate neighbourhood of the wound or they may be perceptible at a great distance from it. The influence may last only a short time after the injury or for many weeks or months (Kuster, 1925).

When herbaceous plants are injured the cells at the border of the wound die off. Beneath the dead cells a secondary meristem or wound-meristem may be built, though only mature cells have been wounded (Went, 1923).

Secondary meristems originate from mature living cells, which become meristematic under the influence of a wound. They form a layer of initiating cells amid uninjured parenchymatous tissues adjacent to the wound. The cell-divisions take place parallel to the wound-surface. The newly formed tissue, the wound-periderm, commonly consists of three layers of tissue: the initiating layer or meristem, known as the phellogen or cork-cambium; the layer of cells

formed by this meristem towards the outside, the phellem or cork; and, usually, a layer formed toward the inside, the phelloderm. (Eames & Daniels, 1925). The cell-walls of the periderm have a coat, the cork- or suberin-lamellae, consisting of a lipophillic substance (Frey-Wyssling, 1959). One of the special functions of the periderm is the protection of wounds through the production of wound-cork. Tissue dead from any cause is usually shut off from that which is healthy by such a periderm layer (Eames & Daniels, 1925).

Often other processes take place in the living cells adjacent to the wound. The cell-walls of the cells bordering the wound are lignified and cork lamellae are deposited against the walls. These processes, called meta-cutinisation, are very common in plants after wounding. In this way an enclosure of the wound-surface of a potato tuber may be formed as early as 12 hours after injuring.

The initial cells of the secondary meristem may also produce callus-tissue. External conditions are of great influence on the method of wound-healing; if humidity is low, periderm is formed. In that case cell-multiplication progresses slowly, the new cells differentiate soon and the cells just beneath the wound-surface become corky. If humidity is high, hypertrophy and cell-division occurs, the multiplication of the cells proceeds at a high rate and the great mass of parenchymatous or meristematic cells are considered as callus. Only the walls of the cells at the surface of the new tissue are suberized, especially when the outer layers are dying off (Fitting, 1947). There is, however, no clear cut difference between wound-periderm and callus-tissue.

The reversion of mature cells to the juvenile state is ascribed to the action of wound-hormones, produced by the wounded cells (Haberlandt, 1921; Scheer, 1953). Also the intact cells surrounding the wound produce substances of influence on the formation of hypertrophic cells and on the occurrence of cell-divisions. They are produced only during a definite period after injuring. When the wound-surface is rinsed with water for ten hours, no callus-tissue is built afterwards. The cells under the wound-surface are, however, capable of reacting when after the rinsing period, wound-sap is put on the wound-surface. In that case callus-tissue is formed (Braun, 1957).

Wound-hormones present in the wound-sap of plants not only effect the morphology of resting cells, but provoke: 1) an increased respiration (the so called wound-respiration), 2) an increased metabolic activity, 3) changes in the permeability of the protoplast, and 4) an accelerated rate of protoplasmic-streaming. The latter was observed by Zech (1952), who studied the influence of injuring a hair on a tobacco leaf. By cutting the tip-cell of the hair the rate of the protoplasmic-streaming in the other cells was increased immediately after the injury. Fifteen hours later the rate of flow was normal again.

It was shown by Braun (1952) that the condition of the cells beneath the wound-surface is determining for the occurrence of a tumor in the presence of virulent crowngall bacteria, *Agrobacterium tumefaciens* (Sm. et Towns.) Conn. The tumor is formed only when the virulent bacteria come into contact with cells activated by wound-substances.

This "state of conditioning" occurs 24 to 96 hours after wounding. Conditioning takes place gradually, it reaches a maximum during the period from 48 to 72 hours after a wound has been made. The activation is proportional to the concentration of wound-substances. The first 48 hours after wounding seem to be important for the activation of the cells. It is at this stage that the cells show a high rate of metabolic activity. The stage of "conditioning" passes into a state in which microscopically visible wound-reactions occur. After this period the healing process has advanced too far and the bacteria are no longer able to induce tumor-formation.

4.2. Wound-reactions in Nicotiana tabacum L. cultivar. Samsun

It may be asked which changes are effected in the tissues of a tobacco plant, when the axillary buds or the stemtips are removed or when the surface of the stem is slightly wounded.

To answer this question, transverse sections of the wound-region were made at different times after wounding of the stem or removal of the stemtip and the axillary buds. The sections were stained with ammoniacal gentian-violet-solution and differentiated in a hydrochloric acid-solution in water. In this way only suberin- and cork-containing cell-walls were stained. According to Schömmer (1949) this staining is more sensitive than the staining with Sudan III.

Twenty-four hours after wounding of the stem-surface the beginning of the coating of suberin against the inner-side of the walls of the intact cells beneath the wound-surface was perceptible. After 48 hours the suberin-layer was more intensively stained. During the next 72 hours

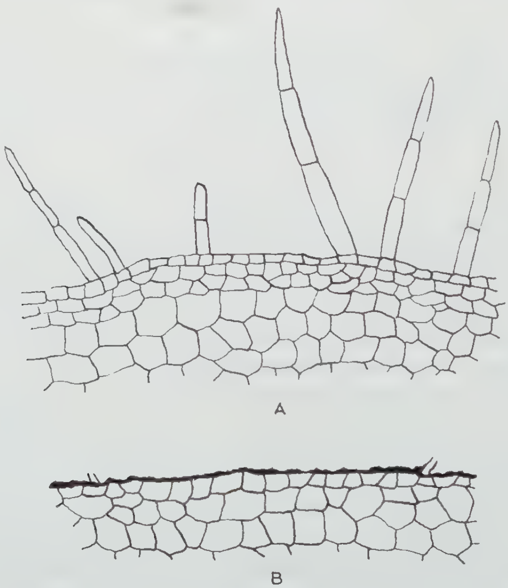

Fig. 2. Transverse section of stems of *Nicotiana tabacum* L. stained with ammoniacal gentian-violet-solution. A: unwounded; B: 48 hours after wounding.

this condition remained unchanged and after this period the observations were stopped.

These results indicate that here a simple process of wound-healing had taken place. The damaged cells died, became brown and parched, and within 48 hours the walls of the underlying cells were coated with a suberin layer. The same process occurred after removal of the stemtip or the axillary buds. The building of a secondary meristem or formation of callus-tissue has never been detected. No microscopically visible changes were observed later than 48 hours after wounding (Fig. 2). There was no difference in the way of wound-healing between the healthy plants and the plants infected with TMV, under the same conditions.

It was considered possible, that during the first 48 hours after wounding, in which period the wound-reactions occur, the active state of the cells is of influence on the direction and the rate of the virus-transportation in the plant. To investigate this possibility, additional experiments were carried out.

4.3. Influence of wounding of the stem on the rate of virus-transportation

In the following experiments, the influence of removing the axillary buds and the stemtip on the rate of virus-transportation was compared with that of wounding of the surface of the stem.

The experiment was carried out with four groups of three plants. In the first group no wounding or removal took place; in the second group all the axillary buds and the stemtips were removed; in the third group a slight circular wound was inflicted, surrounding the stem just under the stemtop; and in the fourth group the stem was slightly damaged by injuring the epidermis along its whole length at two opposite sides. Simultaneously, a middle leaf of all plants was inoculated. The plants were tested for the presence of the virus, 120 hours after the inoculation (Table V A and B).

The virus could not be detected in any of the rootsystems. In the first group the ratio of infected leaves was only 0.35. In the second group the ratio was 0.67, an increase which was in agreement with the results of the preceding experiment (p. 125). In the third group the ratio was found to be 0.43. Wounding of the stem just under the top without removal of the stemtip appears to have as little influence on the virus-transportation in the plant as a wound made by the removal of the stemtip. It is remarkable that here the virus was transported only in upward direction out of the inoculated leaf. In the fourth group the ratio of infected leaves was highest, namely 0.81. Thus it appears that the wounds of the epidermis inflicted along the whole length of the stem have a greater influence on the rate of virus-transportation than the very small circular wound under the stemtop, and somewhat more than removal of all the axillary buds.

In a following experiment this was further confirmed: Twenty-five plants were divided into five groups of five plants, all six weeks old. In the first group no buds were removed or wounds inflicted. In the

TABLE V

Spreading of virus in plants after removal of the stemtip and the axillary buds or wounding of the stem, 120 hours after inoculation of the middle leaf.

A. Place at the stem of the infected leaves.

Group number 1)	1			2			3			4		
Treatment	Controls			Stemtips and axillary buds removed			Circular wound under the stemtop			Wounding of the stem along its whole length		
plant number . . .	1	2	3	1	2	3	1	2	3	1	2	3
										x′		
6th leaf	–′		–′	★	★	★		x′	x′	x	x′	–′
5th ,, ...	–	–′	x	–	–	x	x′	x	–	x	x	x
4th ,, ...	x	–	x	x	x	x	x	–	x	x	x	x
3rd ,, ...	•	x	•	•	•	•	•	•	•	•	x	•
2nd ,, ...	x	•	–	x	–	x	–	–	–	x	•	x
1st ,, ...	–	–	–	x	x	–	–	–	–	–	–	x
rootsystem	–	–	–	–	–	–	–	–	–	–	–	–

B. Number of infected leaves in relation to the total number of leaves per group

Group number 1)	Virus-containing leaves	Ratio 3)	Virus-containing rootsystems
1	5/14 2)	0.35 ± 0.09	0/3
2	8/12	0.67 ± 0.14	0/3
3	6/14	0.43 ± 0.06	0/3
4	13/16	0.81 ± 0.02	0/3

1) Three plants per group.
2) and 3) See note Table I.
Explanation of symbols, see Table II.

second group the stemtips were removed and in the third group all the axillary buds. In the fourth group a small circular wound was made just under the stemtop and in the fifth group the stems were wounded along their whole length at two opposite sides. These treatments took place simultaneously with the inoculation of the middle leaf. One hundred and twenty hours after this treatment the leaves and the rootsystems were cut off and tested for the presence of virus (Table VI A and B).

In this experiment the number of virus-containing leaves was smaller than in the preceding one. However, in all groups the virus had penetrated into the roots. In the controls the ratio of infected leaves was only 0.10. In all the other groups this ratio was higher; in the second group 0.28. Here the virus-transportation took place mainly in an upward direction. In the third group the ratio was 0.55. So here a great virus-spreading had taken place and both upward and downward. In the fourth group a ratio of 0.35 was found; this

Table VI

eading of virus in plants after removal of the stemtip, the axillary buds or wounding of the stem, 12 hours after inoculation of the middle leaf.

Place at the stem of the infected leaves.

roup mber 1)	1					2					3					4					5				
eatment	Controls					Stemtips removed					Axillary buds removed					Circular wound under the stemtop					Wounding of th stem along its whole length				
Plant umber	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
leaf . .		–′				★	★		★																
,, . .		–		x′		x	x	★	x	★	–′	–′	x′	–′	x′	x′	–′	x′	–′	x′	–′	x′	x′	x′	x
,, . .	x′	–	–′	–	–′	–	–	x	–	x	x	x	–	x	–	x	x	–	x	x	x	x	x	x	–
,, . .	–	●	–	●	–	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
d ,, . .	●	–	●	–	●	–	–	–	–	–	–	x	x	x	x	–	–	–	–	–	x	–	–	–	x
,, . .	–	–	–	–	–	–	–	–	–	–	x	x	–	–	–	–	–	–	–	–	–	x	–	x	x
tsystem.	–	x	–	–	x	–	–	–	x	–	–	x	x	–	x	x	–	–	x	–	–	–	–	x	x

B. Number of infected leaves in relation to the total number of leaves per group.

Group number 1)	Virus-containing leaves	Ratio 3)	Virus-containing rootsystems
1	2/18 2)	0.10 ± 0.14	2/5
2	5/18	0.28 ± 0.04	1/5
3	11/20	0.55 ± 0.11	3/5
4	7/20	0.35 ± 0.14	2/5
5	13/20	0.65 ± 0.14	2/5

1) Five plants per group.
2) and 3) See note Table I.
Explanation of symbols, see Table II.

ratio does not differ markedly from that of the second group. These results confirm those of the preceding experiments, which showed little difference between stemtip-removal and circular injury below the stemtop. In the fifth group the virus-spreading was greatest with a ratio of 0.65. These results indicate that both wounding of the stem along the whole length and wounding by removing all the axillary buds accelerate the speed of virus-transportation in a tobacco plant.

It is thus impossible to determine the influence of the stemtips or the axillary buds on the virus-transportation in a plant by removing these plant-parts, for by removing these meristematic tissues, wounds are necessarily made. Probably the wounding, followed by wound-reactions has such an influence on the virus-transportation that a possible effect of the stemtips or the axillary buds cannot be demonstrated.

4.4. Influence of different phases of wound-healing on the rate of virus-transportation

From the preceding experiment it became evident that a wound exercises an attractive action on virus-material present in the plant. Tissues in a phase of wound-healing may influence the speed with which virus moves through a plant.

A question is whether the wound in all the different phases of healing exercises an attractive action. From histological observations in tobacco plants it became evident, that only during the first 48 hours after wounding microscopically visible changes occurred in the cells under the wound-surface (p. 129). Braun (1952) from his experiments with *Agrobacterium tumefaciens* concluded that the wound-reactions occur immediately after injuring and that they last longer than 48 hours. Wounded cells of tomato plants were found to be in an activated state from 24 to 96 hours after wounding. The state of conditioning reached the highest level during the period of 48 to 72 hours after wounding. It is then possible, that abnormal physiological processes caused by wounding, though microscopically not visible may occur also in cells of the tobacco plant, activating them over a period of more than 48 hours (p. 128).

It was therefore important to determine the influence of wounding in tobacco plants on the rate of virus-transport for longer than 48 hours after damaging the epidermis.

In the following experiments the stems of the plants were wounded from 48 hours before to 72 hours after inoculation in order to determine the influence of the different phases of wound-healing on the speed of virus-transportation in a tobacco plant.

The experiments were carried out with seven groups of three plants. Plants of one group served as a control and were not wounded. Those of the other six groups were wounded by injuring the stem along its whole length at two opposite sides, 48 and 24 hours before, simultaneously with, 24, 48 and 72 hours after the inoculation of the middle leaf, respectively. The leaves were tested for the presence of virus 96 hours after the inoculation in the manner described (Table VII).

It became evident that the number of virus-containing leaves of the control plants did not differ markedly from the plants, wounded 48 hours before the inoculation; the ratio being 0.24 and 0.27, respectively. Probably the cells surrounding the injured area of the plants wounded 48 hours before inoculation were no longer in an activated state. Wound-healing may have been completed or nearly so. The ratio of 0.35 which was found in plants wounded 24 hours before inoculation, is of only slight statistical significance in differing from the control plants. When virus-transportation started in these plants, the cells in the neighbourhood of the wound were possibly no longer in the period of optimal activity. The activity was, however, still great enough to cause a very slight increase in the rate of virus-transport. Wounding simultaneously with, or 24 hours after, the inoculation resulted in an increased rate of virus-transportation, as

TABLE VII

Spreading of virus in plants, 96 hours after inoculation of the middle leaf and after wounding of the stem along the whole length at different times.

Experiment [1])	Moment of wounding with respect to the moment of inoculation	Virus-containing leaves	Ratio [3])	Virus-containing rootsystems
A	controls, not wounded . . .	4/16 [2])	0.24 ± 0.07	0/3
B	48 hours before inoculation .	5/18	0.27 ± 0.10	0/3
C	24 ,, ,, ,, .	6/17	0.35 ± 0.04	0/3
D	simultaneously with ,, .	8/18	0.44 ± 0.10	0/3
E	24 hours after ,, .	8/17	0.47 ± 0.06	1/3
F	48 ,, ,, ,, .	6/16	0.38 ± 0.04	0/3
G	72 ,, ,, ,, .	4/17	0.23 ± 0.09	0/3

[1]) Three plants per group.
[2]) and [3]) See note Table I.

the ratios were 0.44 and 0.47, respectively, 96 hours after inoculation. The tissues were probably in a period of optimal activity at the time the virus-material reached the attraction-sphere of the wounds. In plants wounded 48 hours after inoculation with a ratio of 0.38 the influence of the injuries was smaller, but significant different from that of the control plants. The plants wounded 72 hours after inoculation showed a ratio of 0.23, not differing from the controls. In this experiment possibly the virus-material had already started to move through the plant before the injuries were inflicted.

From these experiments it can be concluded that wounding seems to influence the speed of virus-transportation only when injuries are made within the period 24 hours before to 48 hours after inoculation of a middle leaf.

It may be asked, how much time the virus-material needs to enter the attraction-sphere of a wound of the stem after a leaf has been infected with the virus.

No virus can be demonstrated during the latent period, the first 30 hours following inoculation of a tobacco leaf with TMV. Virus-multiplication begins probably six to seven hours after the establishment of the infection (SIEGEL c.s., 1957; YARWOOD, 1952; KASSANIS, 1959). During the latent period, the virus-concentration is probably lower than 10^{-16} gram TMV/ml (SCHRAMM c.s., 1958).

In the literature, different data are found concerning the moment at which the newly built virus-particles start moving out of the inoculated leaf and reach other parts of the plant. Several authors have shown that the TMV or the infectious agents seldom begin to move out of an infected leaf earlier than 48 hours after inoculation (CAPOOR, 1949; KUNKEL, 1939; BEEMSTER, 1958). This was confirmed in our experiments (p. 123). It seems then reasonable to assume that virus-material enters the attraction-sphere of wounds approximately 48 hours after inoculation.

The wounded cells seem to influence the rate of virus-transportation

out of the inoculated leaf only between the moment of wounding and somewhat over 72 hours afterwards. This period coincides with the period of optimal activity of the conditioned cells mentioned by BRAUN (1952, BRANTS, 1961).

These experiments show that not only cells of tomato plants, but also those of tobacco plants are activated by wounding, though this fact has not been confirmed by microscopical observations. Also Braun could not observe microscopical or cytochemical differences between normal and conditioned cells.

It may be asked, what influence the removal of the axillary buds has on the rate of virus-transportation in a plant, when the removal is made at different times with respect to the moment of inoculation of a middle leaf.

In order to determine the effect both of removal of the axillary buds and of the wounding made by this removal, the following experiments were carried out. Twenty-one plants were divided in seven groups of three plants. One group served as a control and no axillary buds were cut out. In the other six groups all the axillary buds were removed at different times with respect to the moment of inoculation of a middle leaf, namely 48 and 24 hours before, simultaneously with, and 24, 48 or 72 hours after inoculation, respectively. The plants were tested for the presence of virus 96 hours after the inoculation (Table VIII).

TABLE VIII

Spreading of virus, 96 hours after inoculation of the middle leaf and after removal of the axillary buds of the plants at different times.

Experiment [1]	Moment of wounding with respect to the moment of inoculation	Virus-containing leaves	Ratio [3]	Virus-containing rootsystems
A	controls, not wounded . . .	4/14 [2]	0.28 ± 0.10	0/3
B	48 hours before inoculation .	4/13	0.31 ± 0.09	0/3
C	24 ,, ,, ,, .	6/12	0.50 ± 0.00	0/3
D	simultaneously with ,, .	10/14	0.72 ± 0.10	1/3
E	24 hours after ,, .	9/14	0.65 ± 0.09	1/3
F	48 ,, ,, ,, .	7/12	0.58 ± 0.14	0/3
G	72 ,, ,, ,, .	4/14	0.28 ± 0.10	0/3

1) Three plants per group.
2) and 3) See note Table I.

The ratio of virus-containing leaves of the control plants (0.28) did not differ from that of the plants from which the axillary buds were removed 48 hours before inoculation (0.31). From the preceding experiment it appeared that wounding 48 hours before inoculation produced no perceptible effect (Table VII) and thus it may be assumed that the absence of the axillary buds had no effect either. Removal of the axillary buds within a period of 24 hours before to 48 hours after inoculation caused an increased rate of virus-transporta-

tion from the inoculated leaf to the other leaves, with a maximum when removal took place simultaneously with inoculation.

Results of these experiments agreed with the results of the previous experiments in which the stem was wounded. Again the highest increase of the rate of virus-transport occurred when the beginning of transportation (48 hours after inoculation) coincided with the period

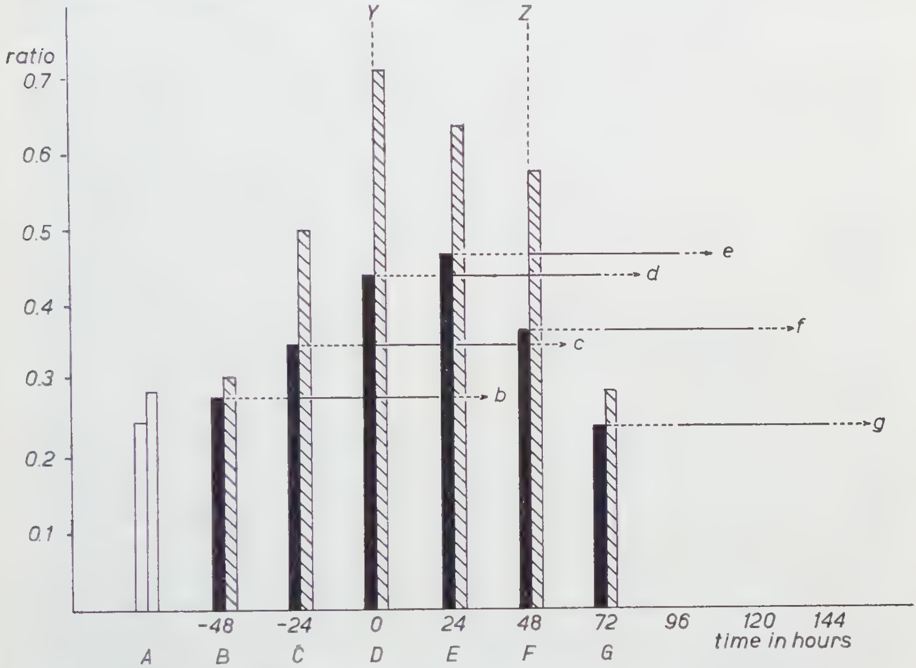

Fig. 3. Effect of wounding at different times with respect to the moment of inoculation on the rate of virus-transport. Abscis: time in hours with respect to the moment of inoculation. Ordinate: ratio of virus-containing leaves. Y: moment of inoculation. Z: begin of virus-transport out of the inoculated leaf.

□ : ratio of virus-containing leaves in plants without wounding or removal of axillary buds.

■ : ratio of virus-containing leaves after wounding of the stem.

▧ : ratio of virus-containing leaves after removal of the axillary buds.

——→ : period of conditioning of the experiment concerned.

of optimal activity of the wounded cells. The trials showed that at least in this experiment removal of the axillary buds had a greater influence than wounding of the stem (Fig. 3). It is likely, that injuries inflicted over a great surface or upon many places have a greater influence on the rate of virus-transportation than small wounds and that it was not the absence of the buds but the wounding that caused these effects. So removal of all the axillary buds or wounding the stem along the whole length will have a greater influence than removing a stemtip or a circular wounding of the stem under the top.

From the last two groups of experiments it can be concluded that wounding with or without removal of the axillary buds influences the beginning of the virus-transportation out of the inoculated leaf only when the injury is inflicted within a period of 24 hours before to 48 hours after inoculation. It is unknown whether there is an increased food-transportation in the direction of the wounded cells during the conditioning phase. If this does occur, an increase in virus-transportation may be expected if virus-material moves together with nutrients. Healed wounds would not exert this influence.

It appears probable that the influence of a wounded area is limited to a local action, since removal of the stemtip did not influence the rate of virus-transportation. Therefore it was desirable to determine the influence of cells in a state of conditioning caused by localized wounding. The following experiments were performed with leaves in order to investigate the extent of this influence.

5. INFLUENCE OF WOUNDING ON THE RATE OF VIRUS-TRANSPORTATION IN A TOBACCO LEAF

5.1. Literature

Zech (1952) has investigated extensively the spread of virus in an inoculated leaf. The increased rate of streaming of the protoplasm, occurring in the cells after inoculation of a hair, was considered to be an indication that the virus or the infectious agent had reached these cells. In leaf-hairs wounded but not inoculated with virus, an increased rate of protoplasm-streaming was also observed, but became imperceptible after 20 hours. It can be asked, however, whether this accelerated speed of streaming is really an indication for the rate of spreading of the virus-material, as it is possible that this material passes through the cells without causing microscopically visible changes. Zech assumes that only after the passage of non-infective precursors of the virus-nucleoproteins, or other substances, streaming of the protoplasm in the cells is accelerated. He observed that six hours after inoculation the protoplasm-streaming in the upper- and under-epidermal cells was increased. In the upper-epidermis the infection first extended slowly to all sides. When the epidermal cells lying over the nerves were reached, the extension took place faster, parallel with the nerves. In a direction perpendicular to the veins, the rate of virus-transportation was about five times less. The infection in the parenchyma of a leaf extended first in all directions just as in the upper-epidermis. Virus-material follows a nerve in apical and basal direction, but more quickly in basipetal direction. During the first two days after inoculation, no infectious material was detectable in the leaf by means of the local lesion test on *Nicotiana glutinosa* L. The third day after inoculation the first inclusion bodies appeared and virus-material could be demonstrated in the expressed leaf-sap. From the fourth day on the number of local lesions increased.

To determine the effect of another kind of wounding, squeezing

the leaf cells, on the number of local lesions formed after inoculation with virus, YARWOOD (1953) pressed bean leaves at 20–240 lbs of pressure per square inch immediately before or immediately after inoculation with TMV. This treatment increased the number of local lesions, whether or not carborundum was used in the inoculation process. The increased infection due to pressing was greatest in leaves which had become somewhat resistant due to age. No effect on infection was observed if the leaves were pressed a day or more before or after inoculation. Inoculation by spraying with a virus-suspension without carborundum was successful immediately after pressing the leaf, but not without pressing. From these experiments it may be concluded that wounding of the leaf results in an increased virus-multiplication.

A disadvantage of the use of leaves to determine the rate of virus-transport is the fact that a leaf is not composed of a homogeneous tissue. HOLMES (1932) suggested that TMV does not generally utilize small veins in its movement in leaves of tobacco plants. When cuts through large veins just below the point of infection were made, the infection of the remainder of the plant was delayed for a short time. Leaves in which cuts were made in intervenal tissues but not through large veins showed less delay in transportation. Wounding by cutting big veins influences the rapid transport of virus-material through these nerves, but probably not the slow transportation from cell to cell in the parenchyma and in the epidermis (KÖHLER, 1950). It may be possible, however, that the conditioned cells in a leaf after wounding are capable of influencing virus-transportation in both the leaf-parenchyma and the nerves.

To examine the influence of cells in a stage of conditioning on virus-transportation in a leaf, the following experiments were carried out with tobacco leaves.

5.2. EXPERIMENTS WITH INJURED, INOCULATED LEAVES

The experiments with leaves were carried out to determine the influence of a slight wounding of a part of a tobacco leaf on the rate of virus-transportation inside this leaf after another part of the same leaf had been inoculated. These experiments can be performed with leaves still on the plant, or with cut leaves. The advantage of attached leaves is, that no extra injury at the petiole is made and the transportation in the leaf influenced by artificial wounding of the leaf-surface can be compared with the transport in unwounded leaves. The disadvantage of experiments with leaves in situ is that transportation in the leaf is influenced by factors lying in the plant itself, such as a tendency to basipetal transport or the influence of the axillary bud near the leaf. Operating with cut leaves, though more easy than with leaves in situ, also has disadvantages. An extra wounding of the petiole must be made and the metabolism of a cut leaf is not comparable with that of a leaf still attached to the plant.

First experiments were performed with cut leaves and leaves in situ to investigate whether the results from these experiments would be

comparable. In all of the following experiments the middle leaves of one plant or comparable leaves from different plants, six weeks old, were used. Defined strips two cm wide and perpendicular to the midrib of attached and cut leaves were inoculated with sap containing TMV. For this treatment a strip at the base, in the centre or at the top of the leaf was chosen. Some leaves were wounded by damaging the epidermal cells and hairs of a second two cm wide strip simultaneously with the inoculation. After inoculation the leaves were plunged into soap-water and then rinsed. The rinsing method appeared to be satisfactory, as virus could never be demonstrated (local lesion-test) in the water used for rinsing. Cut leaves underwent the same treatment as the attached leaves. The cut leaves were placed in a tube containing a half strength KNOP-solution. Twenty-four hours after inoculation all leaves were cut perpendicular to the midrib in as many two cm strips as possible. The borders of the wounded and the inoculated areas coincided always with the borders of the strips. After each cutting the knife was disinfected. The leaf-strips were placed in a petri-dish on diluted KNOP-solution where they stayed for the next 96 hours. During this period the leaves remained green and fresh and the virus-material present in the cells at the moment of cutting multiplied and became infectious.

Through the use of this technique, as opposed to that of ZECH (1952), the rate of transportation of virus-material still not infectious in a leaf can be investigated without microscopic examination of the cells. After 96 hours each strip was pressed out, the sap was rubbed on leaves of *Nicotiana glutinosa*, and two days later local lesions were counted. When more than two lesions were present on one leaf it was assumed that the sap had contained virus. Twenty-four hours after inoculation of a strip at the top, the middle or the base of a leaf, the presence of virus-material in the other leaf-strips was ascertained by the method described above. In many cases, the spreading of virus-material out of the inoculated region had taken place. In Fig. 4, each leaf-diagram represents a combination of three leaves, thus, Figs. A1, A2 and A3 show the spread of virus in nine leaves in situ. Figs. B1, B2 and B3 show that of nine cut leaves, inoculated in the same way. In attached leaves the virus-spread 24 hours after inoculation at the top of the leaf seemed to be somewhat less than in cut leaves (A1 and B1). After inoculation of the middle part the same rate of virus-transport was found in both types of leaves (A2 and B2). Cut leaves inoculated at the bases gave no evidence of virus-transportation, while similarly inoculated attached leaves showed virus-spreading comparable to that observed in leaves inoculated at the top (A3 and B3). It was, however, impossible to detect clear cut differences in the rate of transport between cut leaves and leaves in situ. Therefore the experiment illustrated in Figs. C1, C2 and C3, was performed with cut leaves. Leaves were slightly wounded at different places simultaneously with inoculation of the centre strip. Comparison of diagrams B and C shows that wounding resulted in a greater virus-spread, when tested 24 hours after inoculation. The transport occurred

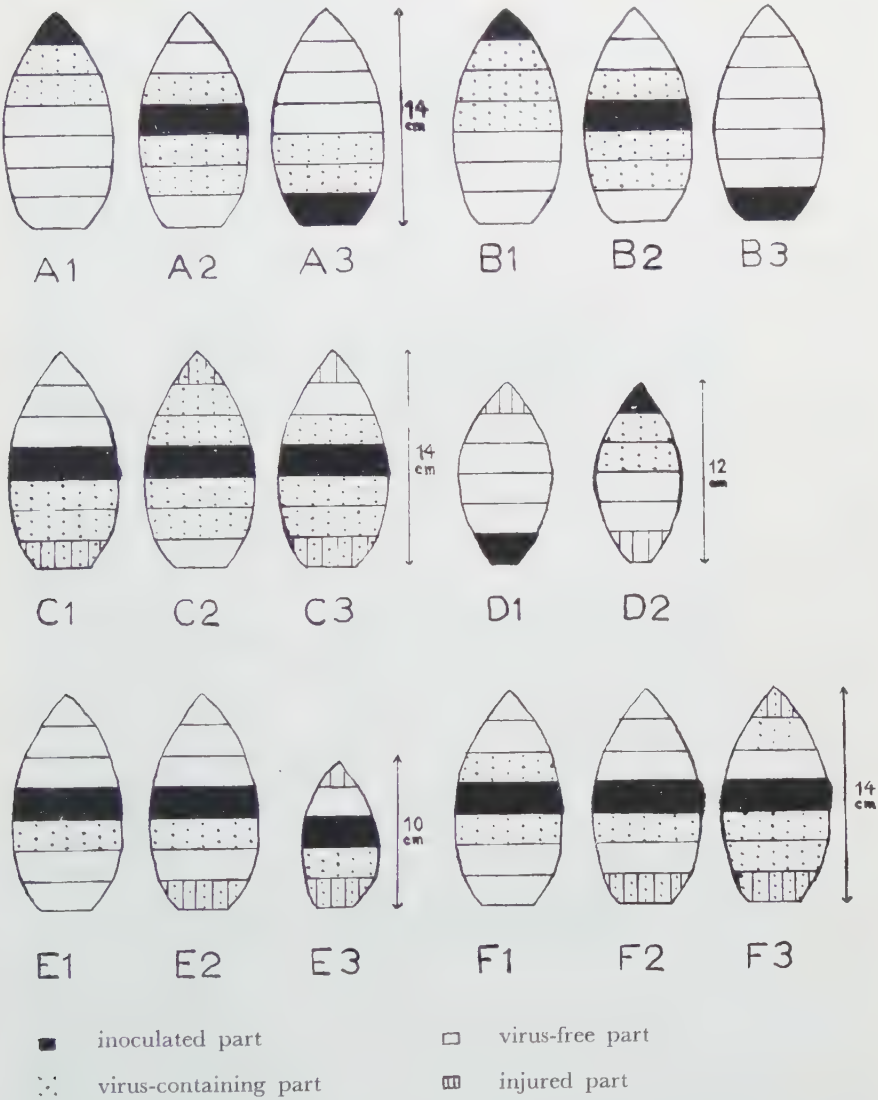

Fig. 4. The way of virus-spreading in leaves. Each diagram represents an average of 3 leaves. A: attached leaves. B to F: cut leaves. A, B, C and D: virus-spreading 24 hours after inoculation. E: spreading after 20 hours and F: spreading 22 hours after inoculation. Injuries were inflicted simultaneously with inoculation.

mainly in the direction of the wound. The effect of wounding at the base seems to surpass the influence of injuring the top (C3). Comparison of diagrams B and D shows that wounding of the top (D1) or of the base (D2) simultaneously with inoculation at the base or at the top, respectively, did not influence transportation of the virus-material. Perhaps the injured area was too far away from the inoculated region to exercise any influence. Since the virus-material was not attracted over great distances and since inoculation of the middle strip resulted in the highest rate of virus-transportation, the middle strip was always chosen as the place of inoculation in the experiments illustrated by diagrams E and F. In both of these experiments one set of three leaves was not wounded, one set was wounded at the base and one was wounded at both top and base. Results were recorded at 20 hours after inoculation in the experiment illustrated in diagram E and that of 22 hours in that represented by diagram F. In all cases wounding caused a more rapid transport of virus-material. In the leaves of diagram E2, F2 and F3 a virus-free region existed between two virus-containing strips. A virus-free region is shown in a diagram only when it was found in at least two of the three leaves in any set. It is possible that the virus-material is transported so quickly along the midrib that no virus-material is left in the virus-free zone between two virus-containing strips. In the former experiments illustrated by diagrams C and D, no interjacent virus-free zones were found 24 hours after inoculation. It may be that such zones are infected by diffusion later as the result of slow penetration of the virus-material in the leaf-parenchyma (Köhler, 1950). In diagram F3, after wounding of both top and base, the virus-material had already reached a great deal of the leaf in the 22 hours after inoculation.

From these experiments it can be assumed that there is some attractive action of a wounded area on virus-material transported in a leaf. It is, however, unknown whether a correlation exists between the period during which virus-material is attracted towards a wound and the period during which the wounded cells are in a state of conditioning. In order to answer this question and, at the same time, to investigate a possible influence of cutting the leaves before treatment, the following experiments were carried out with both attached and cut leaves. It had been found that strips smaller than two cm could be kept fresh during the 96 hours necessary for virus-multiplication and that the width of the strips could be reduced from 2 to 1 ½ cm. In the experiment with cut leaves, the leaves were removed from the plants immediately after inoculation of the middle strip. Injuries were inflicted upon the base of the leaves at 72, 48 and 24 hours before inoculation, respectively, and simultaneously with the inoculation.

These experiments were only partly comparable with the preceding experiments with whole plants, in which stems were wounded from 48 hours before to 72 hours after inoculation. This change in method was essential since it was known that the virus-material could penetrate the whole leaf in 24 hours and in order to detect attraction by the wounded areas, the leaves had to be divided into strips at 20 and 22

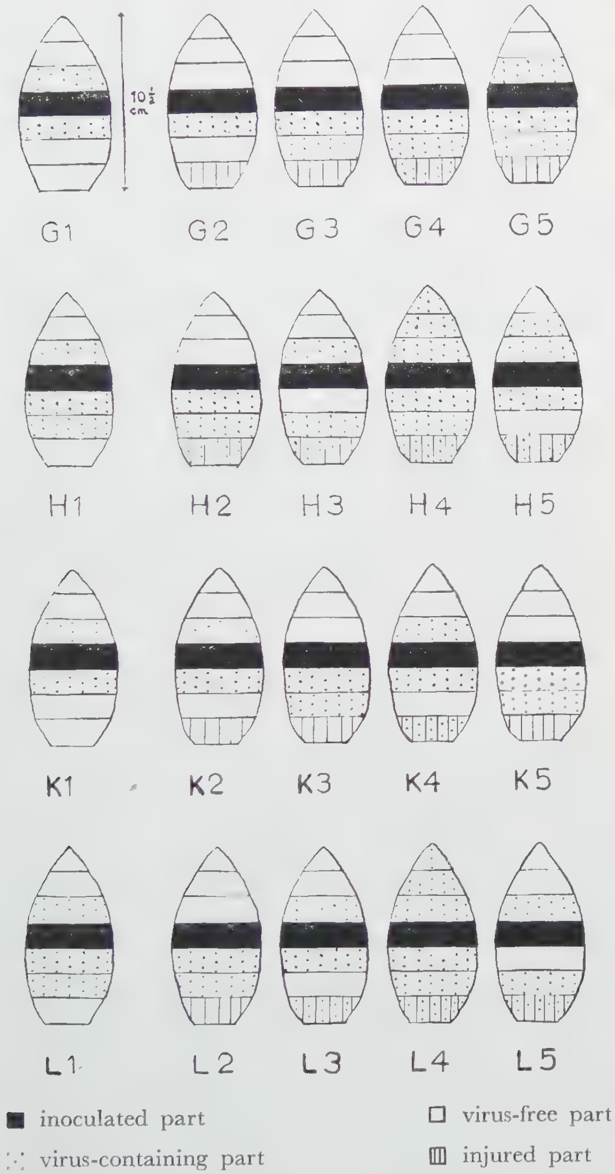

Fig. 5. The way of virus-spreading in leaves. Each diagram represents an average of 2 leaves. G and H: attached leaves. K and L: cut leaves. G and K: virus-spreading 20 hours after inoculation. H and L: virus-spreading 22 hours after inoculation. Numbers 1: unwounded leaves; numbers 2, 3, 4, and 5, in order, injuries inflicted 72, 48 and 24 hours before and simultaneously with inoculation.

hours after inoculation or even earlier. The experiments were performed in duplicate and each diagram in Fig. 5 represents an average of two leaves. The leaves represented by G and H of this figure were leaves in situ; those of K and L, cut leaves. Diagrams G and K represent the results obtained at 20 hours after inoculation; diagrams H and L the results at 22 hours. The numbers 1 to 5 in each series of diagrams refer, in order, to the following treatments: uninjured controls, wounded at the base 72, 48 and 24 hours before and simultaneously with inoculation. In the experiment with attached leaves, only the leaves wounded 24 hours before inoculation contained virus-material in the wounded strip 20 hours after inoculation (G4). Both this treatment and wounding simultaneously with inoculation (G5) caused a more extensive virus-spreading than that obtained in the control leaves (G1). Twenty-two hours after inoculation a greater virus-transportation had taken place: all of the wounded leaves contained virus in the injured base (H2, H3, H4 and H5) while the bases of the control leaves were still virus-free (H1). In comparison with the uninjured leaves only wounding of a leaf-base 24 hours before or simultaneously with the inoculation influenced the virus-spreading markedly. In all the leaves of diagrams H3 and H5, a virus-free region between two virus-containing strips was demonstrated. A zone was considered to be virus-free if the number of local lesions caused by the sap of the strip was less than three. If such a zone was found in the corresponding strips of the two leaves, a virus-free zone was denoted in the scheme. In a few cases only one of the corresponding strips was virus-free. Then the total number of local lesions had to be less than 10 before the two corresponding strips were denoted as virus-free.

In experiments with cut leaves (K and L) nearly the same results were obtained at 20 and 22 hours after inoculation. Wounding of the leaf-base 72 hours before inoculation had no influence on the rate of transportation of virus-material at either 20 or 22 hours after inoculation. The wounded area was not reached by the virus-material at 20 hours. Injuring 48 hours before inoculation exercised the same effect in cut leaves as in attached leaves. The wounded area was reached by the virus-material 22 hours after inoculation. Wounding 24 hours before inoculation had the greatest influence on the rate of transportation. Twenty hours after inoculation all wounded parts were invaded. Wounding simultaneously with the inoculation increased the rate of virus-extension also as compared with the control leaves. Intervening virus-free zones were found in all leaves shown in diagrams K4, L3 and L5, indicating that there is a tendency for the wounded leaf-base to attract virus-material.

From the experiments with inoculated and wounded leaves it can be concluded that the spreading of virus-material in a leaf is influenced by wounding of the leaf-surface only when the injuries are made within the 48 hours-period before the inoculation. To ascertain the moment at which the virus-material begins to leave the inoculated region, the following experiment was carried out.

In six uninjured leaves of which three were attached to the plant and three excised, the spreading of virus-material out of the inoculated middle zone to the other strips was ascertained 18 hours after inoculation. In all cases transportation to one of the adjacent strips had taken place, but the number of local lesions, on *Nicotiana glutinosa* leaves, caused by the sap pressed out of these strips was small: 10 at the most. Therefore it may be assumed that the transportation of virus-material out of the inoculated zone did not start much earlier than 18 hours after inoculation. When injuries are inflicted 48 or 24 hours before inoculation, the conditioning period of the wounded cells will have lasted 48 plus 18 or 24 plus 18 hours, i.e. 66 or 42 hours before transport of virus-material started out of the inoculated strip. It may be assumed that the cells in the wounded region are in a state of optimal activity at the moment that the transportation begins. Wounding of the leaves 24 hours before inoculation caused the greatest virus-extension. Probably the conditioned cells are in a high state of activity about 42 hours after wounding. The influence of wounding, inflicted simultaneously with the inoculation appeared to be smaller and could be explainable by the fact that the wounded cells require some time to become activated.

From the experiments shown in Fig. 3 it became clear that wounded cells in stems demonstrated their optimal activity between 24 and 48 hours after wounding. Results of the experiments with wounded leaves support this conclusion.

6. THE RATE OF VIRUS-TRANSPORTATION IN WOUNDED AND UNWOUNDED PLANTS IN WHICH THE MERISTEMS WERE INHIBITED IN THEIR ACTIVITY

In the experiments so far described, the influence of the stemtips and the axillary buds on the virus-transportation has been determined by removing these meristematic parts. The effect of the absence of the meristematic tissues, however, could not be stated as the wounds made by their removal were of great influence on the rate of virus-transportation. Only the action of the activated cells at the wound-surface could be investigated. We, therefore, tried to determine the influence of stemtips and axillary buds on the rate of virus-transportation by eliminating their action without wounding. This was done by treating the stemtip and the axillary buds with fusarex, 2, 3, 5, 6; -tetra-chloro-nitrobenzene.

Five, six weeks old, plants were powdered lightly with this substance simultaneously with the inoculation of the middle leaf with sap containing TMV. As controls, five plants were inoculated with TMV but not powdered with fusarex. An additional five plants were treated only with fusarex. Fusarex completely inhibited the growth of the plants for about ten days; the activity of the meristems seemed to be eliminated and the plants remained in the same stage of development, which had been reached at the moment of inoculation. Ninety-six

hours after inoculation all the leaves were cut, placed in water and after three days tested for the presence of virus with the method described. The rootsystems were also tested (Table IX).

TABLE IX

Spreading of virus 96 hours after inoculation of the middle leaf and treatment of the tips and the axillary buds of the plants with fusarex.

Treatment	Virus-containing leaves	Ratio [3])	Virus-containing rootsystems
— [1])	6/25 [2])	0.24 ± 0.08	1/5
fusarex	1/25	0.04 ± 0.08	0/5

[1]) Five plants per group.
[2]) and [3]) See note Table I.

Results showed that only one uninoculated leaf from the five fusarex-treated plants contained virus. In the plants treated only with TMV, the virus had penetrated into one or two leaves on each plant in all cases. The treatment with fusarex had obviously inhibited almost all virus-transportation in addition to the meristematic activity. Plants treated with fusarex only, started further development about ten days after the treatment. The new growth showed a slight winding and sometimes a yellowing. Fusarex had apparently exercised a phytotoxic action. In the preceding experiments it was shown that cells near a wound are in an active state and accelerate the virus-transportation in a plant. Since fusarex-treatment appeared to have an inhibiting action on the transport through a plant, it can be asked, what the combined effect of a wound and fusarex-treatment would be. It may be possible, that the effect of wounding surpasses that of fusarex in the plant.

Therefore, in the following experiment the epidermis of the stem was slightly damaged along the whole length at two opposite sides. The trial was carried out with six groups of three plants, six weeks old, treated as follows:

1) all axillary buds and stemtips were powdered with fusarex, 24 hours before inoculation of the middle leaf and wounding of the stem.
2) fusarex-treatment, wounding and inoculation took place simultaneously.
3) the buds and stemtips were powdered with fusarex 24 hours after inoculation and wounding.
4) no fusarex was used, the stem was wounded simultaneously with inoculation of the middle leaf.
5) fusarex-treatment and inoculation occurred simultaneously, no injuries were made.
6) fusarex-treatment and inoculation occurred simultaneously, stems were not wounded and growth of the plants was observed following recovery of fusarex-treatment.

D. H. BRANTS:

The influence of meristematic tissue and injuries on the transport of tobacco mosaic virus in Nicotiana tabacum L. cultivar. Samsun

Plate 1. A: tobacco leaves of one plant in test-tubes containing water 3 days after cutting (1/2 ×); B: tobacco plant from above (3/4 ×); C: stemtop of *Nicotiana tabacum* showing 3 young leaves (3 ×).

Plate 2. A: Healthy callus-tissue of *Nicotiana tabacum* (3 ×); B: Growing stemtip on callus-tissue 15 days after placing (2½ ×); C: Growing stemtip on callus-tissue 15 days after placing showing circumvallation of callus-tissue round the stembase (2½ ×).

The leaves and the rootsystems of the five groups were cut 96 hours after inoculation and then tested for the presence of virus as usual.

It became evident that virus-transportation had taken place only in the plants of the group not treated with fusarex. Five of the 17 tested leaves and two of the three root-systems were infected with virus. In all of the other groups none of the uninoculated leaves or root-systems contained virus. In eight of the twelve fusarex-treated plants, even the sap, pressed out of the inoculated leaves, did not produce local lesions on the leaves of *Nicotiana glutinosa*, even though the sap used for inoculation had shown a high concentration of virus when it was tested on Xanthi tobacco. The leaves of *Nicotiana glutinosa* used for the assay are known to have been in a good condition, since the sap pressed out of the leaves of the group not treated with fusarex, caused the normal number of local lesions.

It was shown that cells in a state of conditioning had not been able to reduce the effect of fusarex, since microscopically visible wound-reactions were the same for both the treated and untreated plants. It may be assumed from this experiment that fusarex prevents or inhibits virus-multiplication in the inoculated leaf to such a degree that subsequent transportation is impossible. The plants of the sixth group treated with fusarex and inoculated with TMV, showed virus-symptoms in the young leaves five weeks after the treatment. The inhibiting action of fusarex on virus-multiplication and transportation seemed to be only temporary. The purely negative results of this experiment made it impossible to determine whether there is a causal relationship between the absence of meristematic activity and the lack of virus-multiplication and transportation. It is possible that a small multiplication occurs, but that inhibition of food-transport causes not only stagnation in development of embryonic tissues but also cessation of virus-transportation if this is dependent upon the streaming of nutrients. The symptoms, shown by fusarex-treated plants, would indicate that damage of tissues in an active state of development would prevent not only virus-transportation, but also virus-multiplication in the leaves.

7. CALLUS-TISSUES

7.1. Introduction

A direct influence of meristematic tissue on the rate of virus-transportation in a tobacco plant has not been demonstrated by the preceding experiments.

Removal of meristematic tissues leads to injuries, which themselves influence the rate of virus-transportation. Inactivation of the meristems by treatment with fusarex seemed to inhibit virus-multiplication. Therefore, it was desirable to find another method to investigate the influence of meristematic tissue on the rate of virus-transportation. The use of callus-cultures seemed to be promising. If it were possible to introduce an active meristem or stemtip into an unwounded callus culture, its influence on the rate of virus-transportation within this

tissue might be determined. The fact that callus consists of homogeneous or nearly homogeneous tissue without vessels in which virus-transportation takes place only from cell to cell, would be another advantage. This may not have been the case in the leaves used in former experiments. Moreover, virus-transportation occurs only slowly in callus-tissue and an accelerating action of a meristem should be readily demonstrated. Considering these advantages callus cultures of tobacco were cultivated in vitro.

7.2. Literature

Much is known about the techniques of the cultivation of tissues in vitro. Practically each kind of tissue requires another medium (Gautheret, 1959). Several precautions are necessary in the cultivation of an homogeneous undifferentiated tissue, since certain substances added to the substratum are able to change the properties of the tissue. Auxins often cause differentiation, followed by stagnation in development and ultimate death of the cultures. Auxin added to the medium in concentration greater than 10^{-8} may induce the desorganized formation of vascular elements in the callus-tissue of tobacco. Virus-transportation over short distances will occur through these elements more rapidly than in homogeneous tissue. Since only undifferentiated homogeneous callus-tissue could be used for our purposes, callus-tissues of tobacco were cultivated on a medium containing auxins in concentrations of 10^{-8} (Morel, 1948). On this medium only a few or no vascular elements are formed in the tissue.

Camus (1949) studied intensively the influence of buds on the morphogenesis of callus-tissue of roots of *Scorzonera hispanica* L. Stemtops of these plants with a length of two to four cm, containing the top-meristem, the leaf-primordia and young leaves, grafted on a callus-tissue, inhibited the development and the growth of new buds out of the callus, induced vascular elements within the tissue and root-formation on its basal side. Camus could demonstrate a continuity between the vascular system originating from the grafted sprout and that of the newly formed roots. The histogenic influence exercised by grafted stemtops on the callus culture in vitro was comparable with that exercised by spontaneously developed sprouts in the callus-tissue. Such a spontaneous meristematic formation could be damaged mechanically in such a way that a deformed sprout, composed of irregularly shaped solid meristematic tissue without leaves, developed out of it. This sprout influenced the callus-tissue in the same way as a normal well-developed sprout with leaves. Therefore, Camus concluded that the histogenic influence is not exercised by the leaves of a sprout, but by the meristematic tissue.

To determine the rate of spreading of virus in tissue-cultures it is necessary to start with healthy callus which is inoculated with virus.

Different methods of inoculating callus-tissue with virus are mentioned in the literature. Not all of the methods have been successful. Kassanis, Tinsley & Quak (1958) obtained infected callus-cultures of tobacco by treating them with sap containing TMV and carborun-

dum-powder. BERGMAN & MELCHERS (1959) came to the conclusion that an infection succeeded only after wounding the tissue mechanically. However, WU, HILDEBRANDT & RIKER (1960) who infected callus cultures by shaking the cells in a nutrient solution containing virus, obtained infected callus-tissue without a carborundum-treatment.

The presence of virus in callus-tissue can be demonstrated either serologically or by application of the local lesion test with *Nicotiana glutinosa*. According to SCHRAMM & ENGLER (1958) the latter method appeared to be the more sensitive of the two, since a minimum concentration of 10^{-12} to 10^{-13} gram TMV/ml is detectable. The minimal infective dose appeared to be smallest in plants reacting with systemic symptoms, such as *Nicotiana tabacum* cultivar. Samsun. Using a considerable number of these latter plants, they found a 50 % infection with a dilution of 10^{-16} gram TMV/ml. This dose was equal to ten virus particles.

The concentration of virus in tissue cultures is always low. According to KASSANIS (1957) it is 30 to 40 times lower than in the leaves of a plant. HIRTH & SEGRETAIN (1956) detected quantities 40 to 50 times smaller than in a leaf, demonstrable with difficulty serologically. Inoculation of *Nicotiana tabacum* with the sap pressed out of the callus culture, the most sensitive method of demonstrating TMV in our experiments, proved to be technically impossible because of the great number of plants needed. Since the small amounts of TMV present in callus-tissue can be demonstrated also by the local lesion test, this method was chosen.

The growth of the callus-tissues appeared to be optimal at a temperature of 20° to 36° C and on media of pH 5.0 to 6.5; the virus-activity calculated per unit of tissue-weight was the highest at a temperature of 24° to 28° C and at a pH of 8.4, according to HILDEBRANDT, RIKER and WATERTOR (1954).

The virulence of the virus does not decline during culturing of the tissue (AUGIER DE MONTGREMIER, LIMASSET and MOREL, 1948).

Virus-multiplication per unit weight of tissue-culture was shown to be greatest during the third month after subculturing, when the growth of the culture declined (HIRTH and SEGRETAIN, 1956). An apparent antagonism seems to exist between virus- and cell-multiplication in the callus-tissue (WU, HILDEBRANDT and RIKER, 1960, HIRTH, 1960). According to KASSANIS (1957), however, the virus-concentration under conditioned circumstances remains constant and is not influenced by the growth of the callus.

Transport of TMV in callus-tissue of tobacco occurs at a rate of about one mm a week, according to KASSANIS, TINSLEY and QUAK (1958). This rate is about the same as that in leaf-parenchyma (KASSANIS, 1956).

Unequal growth in callus-tissue has been described by KLEIN (1957). He observed that cell-division takes place solely in definite regions of the superficial layers of the culture. It seems possible that the virus is spreading more quickly in these layers of the callus culture than in the centre. According to WU, HILDEBRANDT and RIKER (1960),

however, the virus-multiplication in these dividing cells should be smaller.

7.3. Material and methods

For the cultivation of callus-tissues in vitro, greenhouse-grown plants of *Nicotiana tabacum* L. cultivar. Samsun, with a length of 50 cm or more were used. Stem-parts of these plants were treated according to the method of Morel (1948) slightly modified. Though the surface of the stems were not contaminated with many micro-organisms, disinfection was still necessary. This had to be done carefully, as the cambial zone is covered by only a thin layer of cortex. Leaves were stripped from the stem, the stem was cut into 30 cm sections; these sections were plunged into 96 % alcohol to remove all air from the surface. The stem-parts were placed in bleaching-liquor (80 g bleaching-powder per l water) with a maximum temperature of 28° C. After 25 minutes the parts were rinsed twice with sterile water and carefully peeled as far as the cambium, plunged into 96 % alcohol once more and placed in sterile water. The stem-parts were then divided into 1½ to 2 cm pieces. In general, only the basal end of the stem-piece, is able to form callus-tissue. As the contact of this end with the substratum often inhibits growth it is necessary that this end be kept in the air above the substratum. Therefore the stem-pieces were placed on the medium with the apical end downward and the base upward.

The substratum, a KNOP-solution half diluted, contained per l distilled water:

$Ca(NO_3)_2 . 4\ H_2O$	0.5 gram
KNO_3	0.125 gram
$MgSO_4 . 7\ H_2O$	0.125 gram
KH_2PO_4	0.125 gram

3 % glucose,
0.7 or 0.8 % washed agar,
1 mg vitamin B_1,

1 cc HELLER-solution, containing per l distilled water:

$FeCl_3 . 6\ H_2O$ . .	1 mg	$CuSO_4 . 5\ H_2O$.	0.03 mg
$ZnSO_4 . 7\ H_2O$ . .	1 mg	$AlCl_3$	0.03 mg
H_3BO_3	1 mg	$NiCl_2 . 6\ H_2O$ . .	0.03 mg
$MnSO_4 . 4\ H_2O$.	0.1 mg	KJ	0.01 mg

0.01 mg I.A.A. or N.A.A.

5 % coconut-milk, dependent on the condition of the culture.

The tubes with this medium were sterilized for 20 minutes at 108° C. After the stem-pieces were placed in the medium the stoppers were covered with plastic to prevent desiccation of the medium. The atmosphere in the tube was 100 % relative humidity. Unfortunately the percentage of sterile stem-pieces appeared to be low. Bacteria occurred inside the stem-pieces which sometimes developed a month after the pieces were placed into the tubes. The bacteria infected the medium. By adding five cc merthiolate-solution (containing 0.1 % merthiolate and 0.1 % mono-ethanol-amine) per liter medium better

results were obtained, because merthiolate inhibited the growth of bacteria and fungi without influencing the growth of callus-tissue.

At the basal side of the pieces, callus-tissue developed in four to five weeks after the beginning of culturing. This callus-tissue was removed with a sharp knife, under sterile conditions, and placed in another tube of medium of the same composition. The cut surface of the callus was brought into contact with the medium. By subculturing the tissue every four to six weeks, the callus could be kept alive. After this period the volume of the tissue had increased about three times so that the callus culture could be divided into two to four parts. In this way, it was possible to enlarge the number of cultures. After the first subculturing, the tissues were not yet accustomed to life in vitro, growth was often irregular, and the tissues remained in a labile condition. Some of the cultures developed well, others grew badly and died after some time. Results were increasingly better with each subculturing of the vigorously growing cultures, and finally the tissues became accustomed to the medium (Plate 2A). The callus cultures remained growing without differentiation of the tissue when the medium contained a concentration up to 10^{-8} of naphtyl-acetic acid (NAA) or indole-acetic acid (IAA). On one occasion, when the rate of growth declined somehow, for some unknown reason, coconut-milk was added to the medium, and caused recovery of the normal growth rate without inducing differentiation of the tissue.

If stem-pieces from plants infected with TMV and showing distinct virus-symptoms were used for the culture of callus-tissue, the resultant tissue was entirely penetrated by the virus and could not be used for experiments to determine the rate of virus-transportation. We attempted to inoculate healthy tissue-cultures with this material. By placing a slice of the virus-containing tissue in contact with a healthy tobacco callus culture, in more than half of the cases no infection took place. Therefore this method was abandoned.

A more satisfactory method was to inoculate vigorously growing healthy callus-tissue laterally with sterile sap containing TMV and carborundum. At different times after inoculation, slices about one mm thick were cut from the tissue parallel with the inoculated side of the callus. After each cutting the knife was disinfected. It is difficult to obtain slices exactly one mm thick and this irregularity was a source of error since the virus spreads only slowly through the callus-tissue. The sap was expressed by rubbing the slice on a rough glass-slide with the aid of a little glass stick and was tested for the presence of virus by the local lesion test on *Nicotiana glutinosa* leaves. The half-leaf method was used because it is more suitable for indicating presence of small quantities of virus. Although the cells of the cut slices could be kept alive for a short time on a nutrient medium in order to give the virus an opportunity to multiply this method was not satisfactory since the virus-concentration in the cut slices did not increase.

Local lesion tests on the slices disclosed that a sharp border always existed between those slices in which virus was present and those in which no virus could be detected. It is possible, of course, that a very

small amount of virus had penetrated the tissue but insufficient to cause local lesions. One of the difficulties in determining the rate of virus-transportation in callus-tissue is that the rate of growth of the callus is unequal at different sites and this inequality may cause variability in the rate of virus-multiplication. The rate of growth of the inoculated side of the culture usually was much smaller than that of the other sides. This probably resulted from wounds caused by contact with the carborundum-powder at inoculation.

Cultures of virus-infected tissue had the same appearance as healthy ones in both morphological characteristics and anatomical features. The difference between infected and uninfected tissues could be demonstrated only by inoculation of tobacco plants with the sap pressed out of the callus-tissues. This was also established by MOREL (1948) and HIRTH (1960).

The purpose of the experiments was to determine the influence of a sprout with an active meristematic tissue on the rate of virus-transportation in a homogeneous callus culture. For this purpose, well growing callus-tissue laterally infected with TMV was used.

Small sterile stemtips were placed loosely on the callus culture without damaging the tissue. The necessity for avoiding injury of the callus-tissues had become clear from preceding experiments in which wounding had shown a great influence on the rate of virus-transportation.

To obtain sterile stemtips, seeds of *Nicotiana tabacum* L. cultivar. Samsun were exposed to 0.1 % sublimate-alcohol for half a minute, rinsed with water for 30 minutes and placed in a petri-dish with two % water-agar. After ten days they had germinated and the small germs were placed individually on sterile medium of the same composition as that used for the callus cultures. The topmost stemtip containing the top-meristem, leaf-primordia and only one young leaf could be removed from the well developed plant and placed on a callus culture under sterile conditions (Plate 2B and C).

Top-meristems containing at most only one leaf-primordium, as used for so-called "meristem-cultures", were taken from older plants and placed on callus cultures. Only 10 % of these meristems grew out to stems with small leaves and this process sometimes required half a year. Stemtips placed on the callus-tissue on the other hand grew out to a normal sprout with leaves in more than half of the cases (30 of the 55). Therefore most of the experiments were carried out with stemtips although both exercised the same effect on virus-transportation.

7.4. Experiments on the influence of a stemtip on the rate of virus-transportation in callus-tissue

A great number of well-growing callus cultures [1]) were inoculated with TMV at one side. Sterile stemtips were placed on 25 of these tissues simultaneously with inoculation.

Development of the stemtips was visible after ten days. The callus-cells at the surface of the culture enclosed the base of the stemtip and by this circumvallation, the stemtip was held rather firmly on the tissue. Forty days later, no histological connection was found between the stemtip and the callus-tissue.

The rate of virus-transport in callus cultures with and without stemtip was determined at different times after inoculation. Determinations on cultures without stemtip were performed in triplicate at 5, 10, 14, 20, 25, 30 and 40 days after inoculation. Trials with cultures with stemtips were made in duplicate at 10, 20, 30 and 40 days after the inoculation. Callus cultures without stemtips were used as controls in these experiments and, in them, the virus appeared to move continuously through the tissue at a rate of about one mm a week; this agrees with the results of the experiments of Kassanis, Tinsley and Quak (1958) (Table Xa and Xb).

During the first 20 days there was no difference between the rate of virus-transportation in cultures with and without stemtips. After 30 days, however, in both cultures with stemtips, the virus was detectable in slices cut at four mm and six mm from the place of inoculation but not in that slice cut at five mm. Controls of the same age showed no virus beyond the slice cut at four mm. The stemtips also remained virus-free. About four weeks after the introduction of a stemtip, in one case even as soon as 18 days afterwards, rootlets, which penetrated into the medium, became visible at the base of the callus cultures. They appeared to be virus-free. Forty days after inoculation and introduction of a stemtip the virus appeared to have permeated rather suddenly, and to a greater extent, through these cultures than through the controls. Both the stemtip and the rootlets then contained virus.

When a meristem was placed on a callus culture, rootlets also were formed at the base, but it took a long time for this to occur. This delay probably was due to the slow development of the meristem. The cultures without a stemtip or meristem never developed roots.

More rapid virus-transportation in the superficial layers and a somewhat higher rate of spreading in the tissue as a whole would be expected to result from unequal callus-growth. However, virus-concentration in the whole tissue is so small that virus, present in only a few cells at the surface of the tissue, cannot be demonstrated. For that reason it may be possible that the rate of virus-transportation found, is too low. This error would, however, refer only to the superficial cell-layers.

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[1]) I am highly indebted to miss Dra. F. Quak for supplying callus cultures when our tissues were killed by gas.

Table X

A. Spreading of virus in callus-tissue of tobacco; each determination is the result of the corresponding slices of 3 cultures.

Number of days after a one-sided inoculation	Distance in mm from the place of inoculation								
	1 [1]	2	3	4	5	6	7	8	9
5	x	–	–	–	–				
10	x	x	–	–	–				
14	x	x	–	–	–	–			
20	x	x	x	–	–	–	–		
25	x	x	x	x	–	–			
30	x	x	x	x	–	–	–	–	
40	x	x	x	x	x	x [2]	–	–	–

B. Spreading of virus in callus-tissue of tobacco on which a stemtip was introduced; each determination is the result of the corresponding slices of 2 cultures.

Number of days after a one-sided inoculation	Distance in mm from the place of inoculation									
	1 [1]	2	3	4 [3]	5	6	7	8	9	10
10	x	x	–	–	–	–				
20	x	x	x	–	–	–	–			
30	x	x	x	x	–	x	–	–	–	
40	x	x	x	x	x	x	x	x	x	–

x virus-containing slices in all cultures.
– virus-free slices in all cultures.

1) Place of inoculation.
2) Virus present in 2 of the 3 tested slices.
3) Slices with a stemtip.

The sudden increase of the rate of transport in the tissues with a stemtip might be correlated with the appearance of roots. It can be asked which of the anatomical differences occurring in the tissues resulted in this increased rate of virus-transport.

7.5. Anatomy of callus-tissues with and without an introduced stemtip

Cultures were fixed in the fixative of Bouin (30 cc saturated picric acid-solution in water, 10 cc neutral 40 % formol and 2 cc concentrated acetic acid). After 24 hours fixation, the callus-tissues were washed in 80 % alcohol to which lithium-carbonate was added until discoloration occurred, upgraded through alcohol and benzene, and embedded in paraffin mp 58° C. Ten to twenty μ sections were made with the aid of a microtome. They were deparaffinized in xylene and graded down via alcohol and water to the staining-solutions. Langeron's (1942) double staining technique, using methylene-blue and ruthenium-red, was applied. Cellulose in the cell-walls was stained red, woody walls blue-violet.

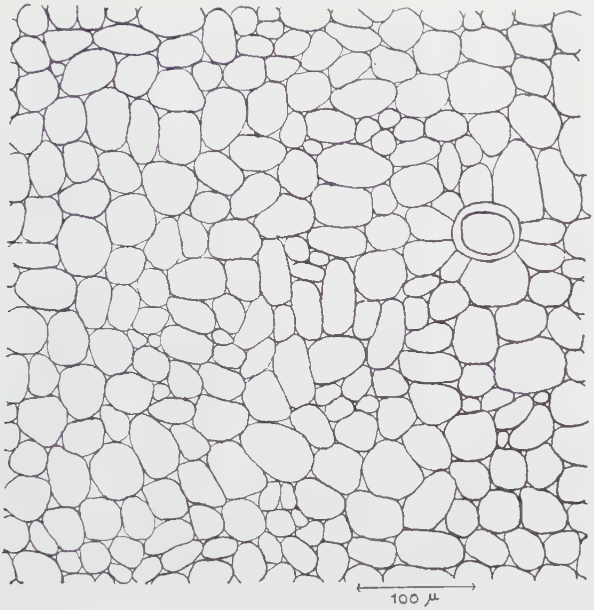

Fig. 6. Callus-tissue of *Nicotiana tabacum*. Undifferentiated tissue.

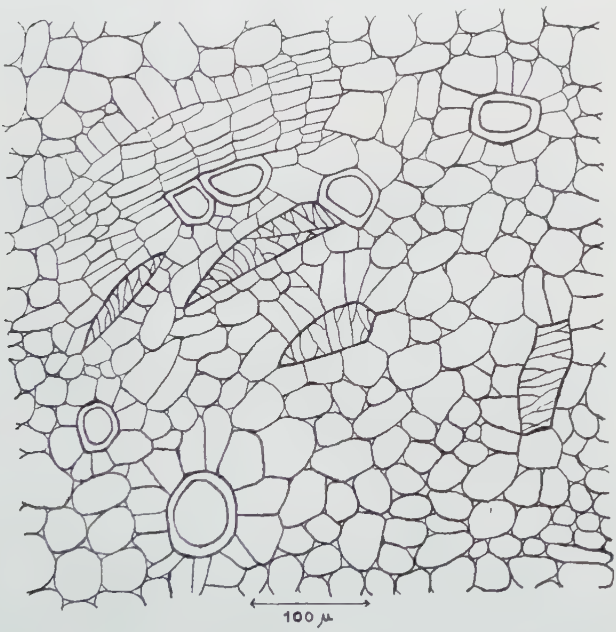

Fig. 7. Callus-tissue of *Nicotiana tabacum*. Occurrence of vascular elements in the tissue four weeks after placing of a stemtip on the callus-culture.

In general callus-tissue without a stemtip showed a homogeneous structure, but, on rare occasions a few vascular elements occurred (Fig. 6). In the tissue on which a stemtip had been placed, well defined changes were observed (Fig. 7). At four weeks vascular elements had developed throughout the whole tissue, and were particularly numerous near the base of the stemtip. In some cases vascular elements in the callus-tissue were oriented towards the vascular strands of the newly formed roots. The structure of these vascular strands in the callus-tissue appeared to be organized.

Introduction of a prepared meristem on the callus-tissue caused the same differentiation of the tissue as that of a stemtip, but was delayed. Although there still was no visible junction between the stemtip or meristem and the underlying callus-tissue, an organization of the tissue took place in both cases. The surface of the callus-tissue under the stemtip or meristem showed healthy cells without a trace of wounding, which were similar to the superficial cell-layers elsewhere. The cells of the cut surface of the stemtip had formed a continuous layer of dried cells and showed the same picture that has already been described for wound-surfaces in normal plants (p. 128). Since the healing-process took place within five days after introduction of the stemtip, it is impossible that wound-reactions resulting from cutting of the stemtip, were responsible for the accelerated virus-transportation which occurred three to four weeks later.

Four weeks after the introduction of a stemtip, transformation and organization of callus-tissue into vascular elements had occurred. It is known that virus-material is transported rapidly through vascular elements and these newly formed directive elements are no doubt responsible for the increased rate of transportation observed in these cultures.

The fact that the virus could not be demonstrated in some sections and that it was present in the adjacent ones indicates that virus-material passed through these sections without leaving any infectious material behind. This phenomenon is well known in stems (Samuel, 1934; Kunkel, 1939; Beemster, 1958) and has been found in our experiments with leaf-parts (Figs. 4 and 5).

The fact of organization makes it unlikely that a stemtip or a meristem, introduced into a callus-tissue, directly influences the rate of virus-transportation in the callus culture. An indirect accelerating influence on the rate of transport is caused by the differentiation induced in the callus-tissue. This is in accordance with the results of Camus (1949), who observed that histogenic changes occurred in callus-tissue of the roots of *Scorzonera* under the influence of a sprout or meristematic tissue.

Although there was no histological connection between the stemtip and the callus-tissue, under influence of this tip the callus began to function as a connection between the stemtip and the newly formed roots, i.e. as a stem with vascular elements. Transportation of material is such a potential plant could occur in a way comparable with that in a normal plant.

The rather sudden increase of the rate of virus-transportation in callus-tissue with a stemtip may be expected at the moment that the vascular elements are formed. The low rate of virus-transportation in undifferentiated callus-tissue can be explained by the absence of communication, with transport occurring only from cell to cell.

The view is widely held that viruses move from cell to cell of leaf-parenchyma through the protoplasmic strands (Sheffield, 1936; Siegel and Wildmann, 1960). As the rate of transportation in callus-tissue is about the same as that in leaf-parenchyma (Kassanis, 1956) it may be expected that callus-tissue also contains protoplasmic strands (plasmodesm). It is however impossible to demonstrate them with the usual fixation- and staining-methods.

Electron photographs (Kassanis, Tinsley and Quak, 1958) have shown that thin places occur in the cell-walls of callus-tissue. These places may play a rôle in the spreading of virus.

To demonstrate the occurrence of plasmodesm, if present, in the callus-tissue the method described by Lambertz (1954) was used. One cm cubes of tissue were fixed in Gilson-solution for 22 hours (40 cc 30 % alcohol, 10 cc concentrated acetic acid, 5 cc 40 % formol and 1 cc 65 % HNO_3, saturated with sublimate), washed in 50 % alcohol, containing jodine. The tissue was cut into sections 25 μ thick with a microtome, the sections were held for five to ten minutes in a 20 % JKJ-solution, stained with sulphuric acid-pyoktannin-solution, rinsed in water, and placed in glycerine for observation under the microscope. In normal pith-tissue of the stem of tobacco treated in this way the plasmodesm were clearly distinguishable. In the cell-walls of the callus-tissue places were visible where the

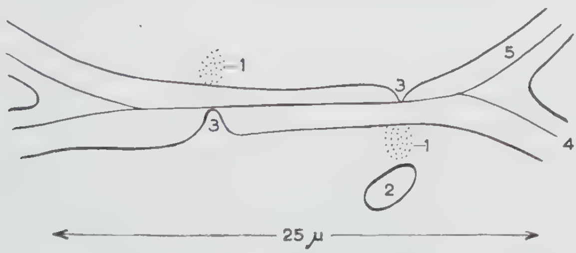

Fig. 8. Cell-walls of callus-tissue of *Nicotiana tabacum* after staining with sulphuric acid-pyoktannin. 1: granulation. 2: nucleus. 3: thin place. 4: middle lamella. 5: cell-wall.

secondary thickening layers were lacking or where they were scarcely present. Opposite these places, at the other side of the middle lamella in the neighbouring cells, a strong granulation in the cytoplasm occurred (Fig. 8). It is possible that at these thin places in the cell-wall an exchange of material takes place through the walls. No real plasmodesm could be demonstrated. The thin places are probably the same as those found by Kassanis, Tinsley and Quak.

8. DISCUSSION

The influence of meristematic tissue on virus-transportation in plants was studied in three different ways. One of the methods used in this respect by several authors was also applied here. The influence of removal of meristematic tissue on the rate of virus-transport was studied. If meristematic tissue attracts virus-material present in the plant, removal of the tissue would cause a change in the rate of transport. To estimate the rate of virus-transport in a plant it was determined in all our experiments at which moment still uninfectious virus-material transported from an inoculated leaf appeared in the uninoculated leaves. For this purpose all leaves of a plant were cut at a definite time after inoculation and kept seperately on water for three days (Plate 1). During this period virus-material, already present in the leaves at the moment of cutting, was allowed to multiply and to become infectious. Then its presence could be demonstrated.

Experiments showed that inoculation of a middle leaf caused the most rapid virus-transport (Table I, II and III). Removal of the stemtip did not influence the rate of virus-transportation out of the inoculated leaf (Fig. 1). Removal of the axillary buds or both axillary buds and stemtip caused an increased rate of virus-transport (Table 4). From these experiments it seemed as if in normal plants the presence of axillary buds exercises an inhibiting action on the rate of virus-transport. However, when the stem was slightly wounded along the whole length without removal of plant-parts, the same result was obtained (Table V and VI). Apparently it was the injury that accelerated virus-transportation in both cases. Therefore remarks mentioned in the literature, suggesting an attractive or a directing action of vegetation-points on virus-transport are doubtful in case these suggestions are based on experiments in which meristematic tissue is removed. At least during 72 hours after this treatment virus-material is attracted by the wounded tissue.

It is known that an injury is followed by wound-reactions, consisting in an increased respiration, an increased rate of streaming of the protoplasm in the cells and coating of the walls with suberin of cells bordering the wound. In some plants wounding may lead to the formation of a wound-meristem and wound-cork.

The reversion of mature cells to the juvenile state is ascribed to the action of wound-hormones which are produced only during a definite period after injuring. According to Braun (1952) the condition of the cells beneath the wound-surface in tomato plants is determining for the occurrence of a tumor in the presence of virulent crowngall bacteria. This "state of conditioning" occurs 24 to 96 hours after wounding with a maximum activity from 48 to 72 hours after a wound has been made.

The influence of cells in an activated condition at the wound-surface of tobacco plants was studied. Experiments were carried out on the effect of wounding at different times in relation to the moment of inoculation on the rate of virus-transport. Influence of the wounded

cells could be demonstrated from 0 to 72 hours after wounding with an optimum from 24 to 48 hours (Fig. 3, Table VII and VIII). The only microscopically visible reaction of tobacco tissue to wounding appeared to be a suberin-coating of cells. This process takes place in the first 48 hours after wounding (Fig. 2). Though after that time no reactions could be observed, physiological activity seems to occur until somewhat over 72 hours after wounding. From these experiments it became evident that cells of tobacco plants are activated after wounding and that these cells attract virus-material reaching the attraction sphere of the wound during the conditioning phase, lasting from the moment of wounding until somewhat over 72 hours afterwards. The duration of the conditioning phase agrees with that determined by Braun, although quite different material and methods were used.

The influence of wounding on the rate of virus-transport was also determined in leaves (Fig. 4). It was possible to check the moment at which virus-material reached a wounded leaf-strip after another part of the leaf was inoculated. Also in leaves, wounds seemed to accelerate the rate of virus-transport. Sometimes virus-material was transported so quickly in the direction of the wound that no material was left in zones between the inoculated and the wounded parts of a leaf. All effects of wounding of leaves supported the conclusions that virus-material appeared to be attracted by wounds during a period from 18 to 66 hours after injuring. Optimal attraction occurred 42 hours after wounding (Fig. 5). The results of the experiments with wounded leaves were in agreement with those obtained from the experiments with plants.

The results of all experiments suggested that wound-reactions influence the rate of virus-transport and it could be concluded that elimination of meristematic tissue by cutting was not a suitable method for studying the influence of this tissue on virus-transport.

The second method applied to investigate the influence of meristems on the rate of virus-transportation was based on elimination of meristem-activity without inflicting a wound. This purpose was reached by powdering the stemtip and the axillary buds of a tobacco plant with fusarex, a compound used for inhibiting the development of sprouts of potato tubers. After this treatment the plants remained in the original stage of development during ten days. When the middle leaf of such a plant was inoculated with TMV a very limited virus-multiplication took place but no virus-transport out of that leaf occurred (Table IX). Ten days after treatment the inhibiting action of fusarex was terminated and the plants started growing again. After five weeks, treated plants showed virus-symptoms. Many leaves were somewhat deformed or discolored showing that fusarex exercised a phytotoxic action. Probably not only the meristem-activity was inhibited by the fusarex-treatment, but also the metabolism of the plant was changed to such an extent that virus-multiplication was impossible and, consequently, nothing could be concluded about the influence of meristems on the rate of virus-transportation. Also in this way no decisive answer was obtained.

From the experiments it can be concluded that cells in an active state attract virus-material. This conclusion is not new since it is known that virus-symptoms first become visible in the young developing leaves, which seem to be suitable for virus-multiplication. It is also known that food-transportation occurs to young cells, as a food-stream will move from the place of food-production, the leaves, to places of food-consumption, such as young tissues or wounds and to places of food-storage. In general nutrients will move from "a source to a sink" (Crafts, 1951). According to Kursanov (1961) the movements of organic substances through the plant are apparently very complicated and the transport of organic materials over long distances is dependent not only on the metabolism of the conducting strands but also on the activities of the organs at either end. Both composition and direction of flow vary considerably for a variety of reasons. Young leaves that have not completed their growth continue for a long time to receive products from mature leaves. Even when they are able to photo-synthesize they do not yield their assimilates to other parts of the plant. These data will explain a great deal of the results of our experiments if it is assumed that virus-transport is correlated with food-transport (Bennett, 1956; Roberts, 1950). It would appear that not the primary meristematic tissue itself, such as vegetation-points, attract virus-material, but the active young tissues formed by the primary meristems, such as leaf-primordia, and mature cells transformed to the juvenile state by wound-hormones. To the actively growing tissues or cells in the "state of conditioning" nutrients and also virus will be transported as they will act as the "sink".

In order to answer the question of direct influence of meristematic tissue itself on the rate of virus-transport, a third series of experiments was performed with tobacco callus-tissue. It was possible to introduce a stemtip of two mm length, cut from a young seedling, or a meristem of about 200 μ, prepared from an older shoot, into the callus-tissue without wounding the culture (Plate 2). The use of callus-cultures made it possible to avoid a disadvantage of the experiments with leaves, in which virus-material is transported in two different ways, a quick transportation through the nerves and a slow transport by diffusion from cell to cell in the parenchyma. Tobacco callus cultures consist of homogeneous tissue in which virus-transport takes place very slowly from cell to cell at a rate of about one mm a week. The rate of virus-transport in a culture could be studied after lateral inoculation with TMV. A further advantage of using tobacco callus-tissue was that only one meristem or stemtip, introduced into the callus-tissue, could influence the rate of virus-transport in the tissue, which is impossible in a plant in possession of many axillary buds. Moreover no injuries had to be inflicted upon the callus-tissue by introduction of the meristematic tissue.

The first 20 days after lateral inoculation with TMV there was no difference in the rate of virus-transport in callus cultures with and without an introduced meristem. Thirty days after inoculation the rate of virus-transport was suddenly increased in the callus cultures

with meristematic tissue in comparison with the controls. Moreover, virus-free parts between two virus-containing regions were found, pointing to an attractive action of the developing meristematic tissue as occurred in wounded leaves (Table X). From these experiments it seemed as if a distinct influence of the introduced meristem on the rate of virus-transport was present. During the 30 days elapsed after introducing a stemtip, the callus cultures had formed rootlets. This was probably due to the influence of the introduced meristematic tissue, since root-formation has never been observed in callus cultures without such a treatment.

Microscopical observations of homogeneous callus-tissue without stemtip showed undifferentiated tissue (Fig. 6). Probably thin places in the cell-walls are responsible for the virus-transport from cell to cell since it has been impossible to demonstrate the presence of plasmodesmata thus far (Fig. 8).

The anatomical differentiations observed in the tobacco callus-tissue on which a stemtip or a meristem had been placed, consisted of the occurrence of vascular elements. The callus-tissue was no longer homogeneous (Fig. 7). No histological connection was found between the stemtip and the underlying callus-tissue. The histogenic influence of the tip must probably be ascribed to an hormonal effect. This is in agreement with the results of the experiments of Camus (1949), who showed that a graft separated from the tissue by a cellophane membrane still caused histogenic changes. It is probable that the formed vascular strands are responsible for the increased rate of virus-transportation in callus-tissue on which a stemtip was placed. The occurrence of virus-free zones between two virus-containing regions suggests that a rapid virus-spreading through the newly formed vascular elements had taken place. Here the meristem did not influence the rate of transport directly, but indirectly by inducing a differentiation of the tissue. The homogeneous callus-mass was changed into a potential plant with a stemtip, vascular tissue and roots.

It could not be demonstrated by all three series of experiments that primary meristematic tissue itself attracts virus-material. The directing action, mentioned in the literature, that has been attributed to top-meristems, apparently does not derive from the meristems themselves, but from the active young tissues formed by the primary meristematic tissues. Thus primary meristems may be considered to influence the rate of transport of virus-material indirectly. Stemtips, introduced on callus cultures, influenced the rate of virus-transport also indirectly by induction of vascular elements in the callus-tissue. Cells in the neighbourhood of a wound, returning to a juvenile state, may be considered as a zone of influence on the rate of virus-transportation during the "conditioning phase".

SUMMARY

1. The influence of meristematic tissue on virus-transportation in a plant was studied in *Nicotiana tabacum* L. cultivar. Samsun, inoculated with tobacco mosaic virus (TMV). The rate of transport of the virus in the plant was estimated by

determining the rapidity with which the virus appeared in uninoculated leaves. For this purpose the leaves of a plant were cut at different times after inoculation of a leaf and placed in water. Under this condition, virus-material present in the leaves at the moment of cutting was allowed to multiply for three days. After this period each leaf was pressed out and tested for the presence of virus by means of the local lesion test on leaves of *Nicotiana glutinosa* L.

2. From experiments in which either the lowest, the middle or the topmost leaf was inoculated, it became evident that inoculation of the middle leaf caused the most rapid virus-transportation.

3. The influence of meristematic tissue on virus-transportation was studied in different ways. One of the most obvious methods is to eliminate the meristematic tissue by removing it. In our experiments removal of the stemtip did not influence either the direction or the rate of virus-transportation out of the inoculated leaf. Removal of the axillary buds or both axillary buds and stemtip caused an increased rate of spreading of the virus.

4. When the stem was slightly wounded without removal of plant-parts the same result was obtained and appearently it was the injury that influenced virus-transportation.

5. Microscopical examination of the wound-reactions in *Nicotiana tabacum* revealed a suberin-coating of cells during the first 48 hours after wounding.

6. It became evident that wounding of the stem of a plant exercised an influence on the rate of virus-transport only when the wound was inflicted 24 hours before, simultaneously with or up to 48 hours after inoculation of a middle leaf. When the injury was made more than 24 hours before or more than 48 hours after inoculation, no influence was perceptible. Probably in the former case wound-reactions had already stopped and, in the latter, the virus-material had already extended too far.

From our experiments it became apparent that 48 hour-incubation of virus-material within a leaf is required before it comes out of the leaf. Then it may be concluded that wounded cells attract virus-material coming out of the inoculated leaf during a period lasting from the moment of wounding up to 24 + 48 i.e. 72 hours after that moment. If the virus-material reached the attraction-sphere of the wound 96 hours after injuring, no influence was perceptible. Optimal attraction by the wounded cells occurred from 24 to 48 hours after wounding.

7. The influence of wounding was also studied in leaves. One part of a leaf was inoculated, another part was wounded slightly at different times in relation to the moment of inoculation. The leaves were cut into sections 20, 22 or 24 hours after inoculation and these strips were kept on a fluid medium for four days in order to allow the virus-material present at the moment of cutting to multiply. Virus-material was transported quickly to the wounded region only when the injuries were made during a period from 48 hours before to simultaneously with the moment of inoculation.

As soon as 18 hours after inoculation virus-material begins to move out of the inoculated region of the leaf. So virus-material seemed to be attracted by wounds 48 + 18, 24 + 18 and 18 hours after wounding, i.e. during a period from 18 to 66 hours after injuring. The attraction was optimal 42 hours after wounding. All effects of wounding of leaves seemed to support the conclusion drawn from the results of experiments with wounded stems of plants.

8. According to Braun wound-activity occurs in a period from 24 to 96 hours with a maximum within 48 to 72 hours after wounding. This period is called the "conditioning phase". His results were obtained from experiments with *Agrobacterium tumefaciens* in wounded tomato plants. The duration of the "conditioning phase" found in these trials agrees with that found in our experiments, in which the effect of wound-activity on the rate of virus-transportation was determined.

9. Another method to investigate the influence of meristems on the rate of virus-transport was elimination of meristem-activity without inflicting a wound. The stemtips and the axillary buds of a tobacco plant were powdered with fusarex (2, 3, 5, 6-tetra-chloro-nitrobenzene), whereupon the plants remained in the original stage of development until ten days after treatment. The action of the meristems was inhibited. When the middle leaf of such a plant was inoculated with TMV a very limited virus-multiplication took place but no virus-transport out of that leaf occurred. Inhibition of meristem-activity and cell-growth diminished virus-multiplication

to a great extent and, consequently, the spread of virus through the plant. After ten days the inhibiting action of fusarex was terminated and the plants started growing again. After five weeks, treated plants showed virus-symptoms, many leaves were somewhat deformed or discolored. Probably not only the meristem-activity was inhibited by the fusarex-treatment but also the metabolism of the plant was changed and virus-multiplication seemed to be impossible.

10. A third series of experiments was performed with tobacco callus-tissue into which a prepared meristem or a stemtip was introduced without provoking injuries. The advantages of this method were:

a) virus-transport could be studied in homogeneous callus-tissue after lateral inoculation with TMV,
b) only one meristem could influence the rate of virus-transport in the callus, which is impossible in a plant in possession of many axillary buds,
c) no injuries were inflicted upon the callus-tissue by introduction of a meristem or a stemtip.

Though the meristems and stemtips developed into small stems with leaves, no histological connection was found between the callus-tissue and the "grafts".

Thirty days after "grafting" the callus-tissue had formed rootlets and the rate of virus-transport was suddenly increased in comparison with the controls without meristem or stemtip.

11. Anatomical observations demonstrated that the stemtip had induced a differentiation in the callus-tissue, consisting of formation of vascular elements. The callus-tissue was no longer homogeneous.

Microscopical observations of homogeneous tissue without stemtip revealed thin places in the cell-walls. Probably these places are responsible for the virus-transport from cell to cell in homogeneous tissue in which virus spreads slowly, about 1 mm a week. Apparently an introduced meristem did not influence the rate of transport in callus-tissue directly, but indirectly by inducing a differentiation of the tissue. The homogeneous callus-mass was changed into a potential plant with a stemtip, vascular tissue and roots allowing transport of material, including virus, in a way, comparable with that of a normal plant.

12. It could not be demonstrated by our experiments that primary meristematic tissue itself attracts virus-material. The directing action mentioned in the literature that has been attributed to topmeristems apparently does not derive from the meristems themselves but from the active young tissues formed by the primary meristematic tissues, such as leaf-primordia, which attract virus. Also mature cells reversed into a juvenile state by wounding exercise an attractive action on virus-material reaching the attraction-sphere of the wound during the "conditioning phase", i.e. during the first 72 hours after injuring.

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THE MORPHOLOGICAL EVALUATION AND TAXONOMIC VALUE OF THE SPATHE IN NAJAS, WITH DESCRIPTIONS OF THREE NEW ASIATIC-MALAYSIAN TAXA

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1. Introduction

In the course of a revision of the genus *Najas* for the Flora Malesiana I was confronted with the morphological evaluation of the spathe and with the variability of its occurrence, because this organ has served for the distinction of sections within the subgenus *Caulinia*.

It appeared of interest to publish the result of this work as a separate precursor as the space available in the Flora will not permit a detailed account.

I have to thank Dr. P. W. Leenhouts for valuable advice and suggestions during the course of the work which was supervised by Prof. Dr. C. G. G. J. van Steenis.

2. Status and taxonomic value of the spathe

Briefly, the gross morphology of a *Najas* plant is as follows. The sheathed leaves on the stem are placed in pseudowhorls of three. On closer examination each whorl consists of 2 subopposite leaves, the sheath of the lowest leaf overlapping the fully amplexicaulous sheath of the upper one. The latter does not produce any shoot from its axil. The lower leaf, however, produces a shoot in its axil which starts immediately with a new similar pair of leaves of which only one leaf (the upper one) develops to full size and forms the third leaf of the pseudowhorl; the other one is reduced to a small scale of microscopical size bearing a bud in its axil. In sterile shoots this bud is merely vegetative, in fertile shoots the scale together with its bud are replaced by a flower, either male or female, which is consequently almost sessile. This structure has been fully explained by Magnus (1870) and has also been agreed to by Rendle (1899).

The male flower consists of only one stamen on a short stalk lengthening in anthesis; this is, however, interpreted as the pedicel, not as the filament, because the anther is entirely surrounded by an adnate, extremely thin envelope ending into two lobes. This envelope is so closely appressed to the anther that it seems to form one whole with the latter, but according to Magnus (1894) it is partly adherent, partly adnate (l.c. t. xi Fig. 3–4). Consequently the envelope is interpreted as the perianth of the flower.

The female flower consists only of a very short-stalked, naked ovary provided with a longish style ending into 2–3 (–4) stigmatic arms. There is no trace of a perianth similar to that in the male flower.

Both male and female flowers may be surrounded by another, but free envelope which has commonly been called the spathe, a thin, membranous, bottle-shaped organ, often obliquely cut at its toothed apex. In the subg. *Najas* (consisting of one variable species only, *N. marina* L.) the spathe is only represented in the male flowers; in subg. *Caulinia* the spathe may occur in both sexes.

The presence or absence of the spathe in one or both sexes has been accepted as of systematical value for a subdivision of subg. *Caulinia.* Rendle has distinguished four sections largely based on its presence or absence, viz sect. *Spathaceae:* both sexes with spathe, sect. *Americanae* and *Euvaginatae:* only males with spathe, and finally sect. *Nudae:* no spathe in either sex.

The morphological interpretation of the spathe has been subject to dispute. RENDLE (1899, p. 383–384) has given a good review of the opinion of various authors about the true nature of the envelopes, i.e. their homology, and their evaluation in comparing the structure as found in *Najas* with that found in affiliated genera or families, such as *Zannichellia*, *Hydrocharitaceae*, *Araceae*, etc. He concluded: "that they found that the homology of the envelopes is difficult to explain on the ordinary terms of definition of the parts of a flower".

Rendle himself accepts the 2-lipped envelope of the male flower as a perianth; the spathe he finds "simply an outgrowth of the axis which ends in the flower below that flower. It is, I think, comparable with the spathe so characteristic of submerged monocotyledonous waterplants, which may have, moreover, a very similar appearance (e.g. *Lagarosiphon*, *Hydrilla*, etc.). It will then correspond, as Magnus suggested, with the cup-like envelope in *Zannichellia*, which on Campbell's interpretation (1897) becomes a spathe surrounding an inflorescence, as in the Aroids."

In passing we mention the very deviating interpretation of Magnus who accepted the "spathe" of *Zannichellia* to contain "ovules" and to be homologous with the ovary in *Najas;* this would lead to the view that the "inflorescence" of *Zannichellia* would represent one gymnospermous ovary. This interpretation is neither accepted by SCHUMANN (1894) nor by Rendle.

The spathe has hitherto generally been accepted as an organ sui generis, save for a casual remark by VENKATESH (1956) who incidentally called it a "bract", without further explaining evidence for that opinion.

It is rather remarkable that Magnus did not pay more attention to the small scale bearing a bud in its axil found at the base of each vegetative shoot connected with the third leaf of each whorl.

Also Rendle, who obviously has considered the possibility that the spathe might be of a leafy nature, expressly stated (1899, p. 382) "that there is no sign of a midrib or of the division of these sac-like outgrowths into leaves."

Najas graminea Del. 1-2. Normal espathaceous ♂ and ♀ flower. 3-4. Spathaceous ♂ and ♀ flower found in one specimen with the normal ones. 5. ♂ Flower with spathe (all ♂ flowers of this specimen, the ♀ were espathaceous).— *N. indica* (Willd.) Cham. 6–7. Espathaceous and spathaceous ♀ flowers of one specimen. 8–9. Spathaceous ♀ flowers, found together with espathaceous ♀ flowers on one specimen. All × 24. — 1–4 Carr 11792 (L), 5 Hook. f. & Th. s.n. (K), 6–7 Gamble s.n. (K), 8–9 Herb. Rottl. (K).

During my work on *Najas* I have seen many hundreds of sheets and examined a couple of thousands of flowers and have come across some specimens of *Najas graminea* Del., a species which normally has naked flowers (Fig. 1–2) but in which occasionally some flowers are provided with an envelope which is intermediary between a spathe and a leaf. Fig. 3–4.

These envelopes have in common with the spathe that they are at least partly closed and have no midrib, and no sheath-like auricles (which are obviously suppressed through the connation of the leaf margins).

They have in common with leaves that they are green, elongated, and possess intravaginal scales as in normal leaves.

Besides I have observed in two specimens from Asia, which are in all other respects agreeing with *N. graminea*, a further stage which nearly approaches that of a normal spathe. In a sheet marked "Ass. Malariol. Publ. Health No. 1" (BM) from Burma one male flower was naked, the three others had a completely closed spathe but with a very thin and relatively long cylindric neck. In another Indian specimen marked "Hook. f. & Th." p.p. (K) all flowers had such a long-necked spathe. Fig. 5.

The variability in occurrence of the spathe just mentioned in *N. graminea* is more clear in *N. indica* (Willd.) Cham. As I have published earlier (1960) the type of *Caulinia indica* Willd. had been erroneously described as being female; all flowers are male and have in this species almost invariably only a spathe in male flowers. In Malaysia the female flower is always naked but in Indian specimens the female flowers have occasionally also a spathe (Fig. 6–9), or more rarely, all female flowers on one specimen are provided with it. Besides, there occur in these occasional specimens intermediary stages between spathe and leaf (Fig. 7, 9), in that the adaxial, spathaceous incision at the apex of the spathe runs down to less than halfway (Fig. 7) or almost to the base of the female flower (Fig. 9). In all I have found 6 sheets of *N. indica* in which spathes are found on female flowers, viz (with an occasional spathe): Madras, leg. Gamble (K), Lawson 12856 (K), Penins. Or. Herb. Rottlerianum (K), and (all ♀ flowers with a spathe): Tranquebar, Soc. Unit. Frat. (BM), Behar, Hook. f. & Th. (K), Sambalpur, Mooney 3785 (K).

A similar occasional development of a spathe I have observed in *N. tenuifolia* R. Br. subsp. *pseudograminea* (W. Koch) De Wilde in which normally the ♂ flower is enclosed by a spathe but the ♀ flower is naked. A collection from Java (leg. C. Schröter & J. H. Coert 454, in L) possessed many ♂ and ♀ flowers. One of the latter flowers was beyond anthesis, and the nearly ripe fruit was enclosed by a spathe with a short neck spathaceously incised to about halfway the neck.

In my opinion these observations can only lead to one conclusion, viz that the spathe of *Najas* is homologous with a normal leaf, namely the lower one of each axillary sessile pair which is in sterile shoots normally developed as a minute scale. This is strengthened by the fact that the intermediary stages just-mentioned, and the normal

spathe are all toothed at the apex as are the leaves. Consequently the flower must be interpreted as axillary.

The observations lead also to another conclusion, viz that the occurrence of a spathe or its absence seems to be of less systematical value than attributed to it in the past and it is my feeling that the distinction of sections based on it is artificial.

In my opinion the distinction of subgenera is also overrated because of the great resemblance of *N. marina* to the other species of *Najas;* I prefer to return to the old opinion of two sections instead of two subgenera.

3. Description of three new taxa of Malaysian Najas

Najas malesiana De Wilde, nov. sp. — *N. bengalensis* Horn af Rantzien, Act. Bot. Gotob. 18: 192, 193. 1950, in clav., descr. angl., ad int., nom. inval. — *N. graminea* var. *minor* Rendle et var. *angustifolia* Rendle, Trans. Linn. Soc. Lond. II, Bot. 5: 426, 427. 1899.

Planta ad c. 15 cm alta, internodiis in partibus inferioribus 1–3 cm × 0,5–0,8 mm. Folia (12–) 19 (–25) mm longa, basi laminae (0,4–) 0,5–0,7 (–0,9) mm lata; lamina plana, apice obtusa vel acutiuscula, circa 3 mm infra apicem 0,25–0,5 mm lata, margine in utroque latere denticulis inconspicuis (20–) 25–30 (–60) munito, denticulis singulis praecipue e spinis compositis, spinis fuscis (0,05–) 0,1 (–0,25) mm longis. Costae latitudo c. 1/20 eius laminae; cavitates septati confines longitudinales latitudinem occupantes usque ad dimidiam partis dimidiatae laminae attingentem. Vaginae, auriculis inclusis, (1,5–) 2–2,5 (–3,0) × 1–2,5 mm, in utroque latere spinulis (2–) 6–14 munitae, margine interiore auriculi spinuli 0–4 praedito. Auriculi elongato-triangulares vel linguiformes, interdum incurvati, 0,5–0,8 (–1,1) mm × 0,3–0,8 mm, integri, raro leviter lobati. Saepe 3 flores ♀ diversae aetatis cum uno flore ♂, masculino femineum appresso, vel ♂ solitarius, floribus masculinis spathella destitutis. Anthera 1-locellata, elliptico-oblonga, interdum apicem versus contracta, 0,6–1,0 × 0,15–0,3 mm; perianthis lobuli saepe inconspicui. Pedicelli 0,2–0,4 mm; in floris maturitate c. 1 mm longi; spathella in floribus ♀ absens. Flores ♀ raro usque ad c. 0,3 mm pedicellati, (1,2–) 1,5 (–1,8) mm longa, ovarium 0,4–0,8 × 0,2–0,3 mm. Stylus 0,25–0,5 mm longus, stigmatibus 2(–1?), 0,3–0,6 mm longis. Semina (0,9–) 1–1,5 × (0,35–) 0,4–0,5 mm usque ad 1,5–1,8 × 0,5–0,6 mm. Testa areolata, areolis subquadrangularibus vel 5–6-angulatis, in seriebus 16–26 longitudinalibus confertis, seriebus e areolis 24–30 compositis.

Holotypus: Sumatra, W. Meijer 5772 (L).

India. East Bengal: Griffith 5609/1 (BM).
Burma. Pegu: Kurz 3310 (BM).
Lower Siam. Setul: Ridley, March 1910 (SING).
Indo-China. Tourane: Clemens 4213 (BM).
Malay Peninsula. Wellesley: in rice fields, Dec. 1895, Ridley (SING), Pahang: Pekan, in pool, 20 Aug. 1889, Ridley (SING), ditto, May 1896, Ridley (BM). Selangor: May 1896, Ridley 7830, doubtful because sterile (SING). Malacca: Griffith s.n. (herb. J. Gay) (K); Griffith 5609/6 (K). Singapore: stream, Tanglin, Febr. 1889, Ridley (SING); Garden lake, 1894, Ridley 8946 (BM).

SUMATRA. Eastcoast Res. Asahan: Sg Piring, 1936, F. Schneider (ZT), ditto, Aek Kwasan, F. Schneider (ZT). Westcoast Res.: Bukittinggi, Karbouwengat (Karbou canyon), W. Meijer 5772 (L).

BORNEO. North Borneo: Gibbs 2820 (BM); Jesselton, Clemens 9699 (BO). Southeast Borneo: Bandjermasin, Motley (BM).

JAVA. Bogor, Inst. Plantenziekten 1 (BO); Tjitjadas, van Steenis 5402.

SOUTH CELEBES. Kendari, Lepo-lepo, Beccari s.n. sheet number 11810A, pro parte (FI); Lasao, Kjellberg 1186 (BO).

PHILIPPINES. Luzon: Manila, Loher 1590 (K); San Francisco del Monte, Loher 1587 (K).

SOUTH MOLUCCAS. Tanimbar Is.: Jamdena, Buwalda 4506 (L).

Notes. The species is easily distinguished from *N. graminea* Del. and other species of the former section *Nudae* by the small, 1-celled anthers and the small seeds.

I have not perpetuated the epithet bengalensis as it has appeared that the species is more abundantly represented in Malaysia.

Because of their poor condition I have not designated as holotype one of the specimens used by Rendle for the description of his varieties. Other good fertile specimens are Singapore, Garden lake, Ridley (BM) and North Borneo, Jesselton, Clemens 9699.

Najas graminea Del. var. **robusta** De Wilde, nov. var.

Planta validior, usque ad 50 cm longa, internodiis inferioribus (1,6–) 2–2,25 mm diam. Cortex caulis e struebus cellularum numerosis compositus, cavitatibus a struebus minime duobus sibi sejunctis. Folia 50–60 mm longa, apud basin laminae 3–4 mm lata, in parte superiore circa 3 mm infra apicem circa 1,2 mm lata, marginibus spinulis inconspicuis 160–185 obsitis. Vagina, auriculis inclusis, (4–) 7,5 (–10,5) × (4–) 6,2 (–8,5) mm, in utroque latere spinulis (15–) 30 (–50) munita. Auriculae elongato-triangulares, subincurvatae, (2–) 4 (–5,5) × (0,8–) 1 (–1,5) mm. Flores (?plerumque) solitarii. Antherae 4-locellatae, ellipticae (vel oblongae), (1,5–) 1,7 – 1,8 (–2,0?) × 0,8–1 mm. Flores ♀ c. 3,5 mm longis; ovarium c. 0,9 × 0,35 mm; stylus c. 1 mm; stigmata c. 1,6 mm longa. Semina ignota.

Holotypus: Ins. Wetar, J. Elbert 4521 (K; isotype L).

Notes. There is no other material known of this variety.

The new variety is clearly distinct from the normal form by the coarse habit, the thick stem, long, wide leaves, etc.

Najas marina L. var. **sumatrana** De Wilde, nov. var.

Planta usque ad 70 cm longa, internodiis in partibus inferioribus 3–7 cm × 0,9–1,4 mm, dense spinulosis (spinulis 15–20 per aream 2 mm metientem, plerisque ad et paulum infra nodos insertis). Folia 35–45 mm longa; lamina ad basin 2–2,3 mm lata, c. 3 mm infra apicem latitudine c. 1,8 mm, plana, tenuis, in sicco translucida, apice acuta vel obtusiuscula, in utroque latere secundum marginem spinulis 30–40 conspicuis, in parte infima (2 mm longa) saepe inermis; foliorum superficies inferior totidem spinulis munita quam margines, in superficie superiore spinulis paucioribus et plerisque spinulis ad costam

confertis, spinulis elongato-conicis 0,75–1 mm longis sursum curvatis, eorum cellula apicali c. 0,15 mm longa fusca cellulis brunneis pluribus inserta. Foliorum vagina 3–3,5 × 4–4,5 mm, in utroque latere secundum marginem spinulis 7–10 munita, superficie inferiore in parte apicali multispinulosa. ♂ Flores ignoti. ♀ Flores 2,5–3 mm longi; ovarium c. 1 × 0,6 mm; stylus 0,6–0,8 × 0,1 mm; stigmata 2 vel 3, c. 1,2 mm longa; semina 4–4,4 × 2,5 mm.

Holotypus: Ins. Sumatra, Lake of Manindjau, E. Jacobson s.n. (L; isotypus BO).

Note. Though it will appear that among the more than one dozen varieties of *N. marina* some may appear to be superfluous, the present one seems to be worthy of distinction. It is clearly distinct by its long, narrow, flat, and thin leaves with very numerous spines along the margin and the midrib; the sheath and stem are similarly densely spinulose.

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ÜBER DEN ENZYMATISCHEN CUTIN-ABBAU

II. MITTEILUNG: EIGENSCHAFTEN EINES CUTINOLYTISCHEN ENZYMS AUS PENICILLIUM SPINULOSUM THOM

WOLFGANG HEINEN

unter technischer Mitarbeit von

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I. EINLEITUNG

Ein wesentlicher Bestandteil der aus verschiedenen Schichten aufgebauten pflanzlichen Kutikula ist das *Cutin*, welches im allgemeinen, auf die Cellulose- und Pectinschicht folgend, die äußere Begrenzung gegenüber der atmosphärischen Umwelt darstellt, sofern man von den aufgelagerten Wachsstrukturen absieht (FREY-WYSSLING 1953, ROELOFSEN 1952, 1959, KOLJO 1957, SITTE 1955, 1957, JUNIPER u. BRADLEY 1958, JUNIPER 1959, BANCHER, HÖLZL u. KLIMA 1960). Die dem Suberin und Sporopollenin verwandte Substanz besteht aus vernetzten, hochpolymeren Fettsäuren oder Oxyfettsäuren (ZETSCHE 1932, FREY-WYSSLING 1953, TREIBER 1955, 1957, MATIC 1956), deren Entstehung aus flüssigen Vorstufen wenngleich nicht bewiesen, so doch zumindest wahrscheinlich gemacht werden konnte (PRIESTLEY 1943, LINSKENS 1950, 1952, SITTE 1955, SIDDIQI u. TAPPEL 1956, BOLLIGER 1959). Bei der Verrottung und Humifizierung pflanzlichen Materials (WIERINGA 1955, FARKASDI 1958, MEYER 1959) tritt offensichtlich auch eine vollständige Destruktion des Cutins ein. Auch verschiedene Beobachtungen beim Eindringen phytopathogener Keime in die Wirtspflanze sprechen für eine dabei eintretende Lyse des Cutins (FULTON 1948, MILLER 1949, YARWOOD 1957, SCHWEIZER 1958, TENERINI u. LOPRIENO 1960). Besonders erwähnenswert ist in diesem Zusammenhang der Befund von LOPRIENO u. TENERINI (1959), daß die durch *Cycloconium oleaginum* verursachten Infektionsstellen bei Olivenblättern vor makroskopischem Erscheinen des Krankheitsbildes dadurch sichtbar gemacht werden können, daß man ein Blatt, bei dem eine Infektion vermutet wird, in eine 5 %ige NaOH von 50–60° C eintaucht. Die Beobachtung, daß sich bei dieser Behandlung an den Penetrationsstellen dunkle Flecken bilden ist wahrscheinlich darauf zurückzuführen, daß dort durch Enzymeinwirkung aus dem Cutin freie verseifbare Fettsäuren entstanden sind. Eine Destruktion des Cutins ist gleichermaßen auch für einen anderen Eindringungsvorgang, das Durchwachsen der Pollenschläuche durch die Narben-

kutikula bei Cruciferen (CHRIST 1959) anzunehmen. Dennoch wurde das Cutin bislang als enzymatisch nicht angreifbares Material angesehen (GÄUMANN 1957, TREIBER 1957, ROELOFSEN 1959). Während MILLER (1949) erstmals die Existenz cutinlösender Enzyme für sehr wahrscheinlich erachtete, konnte kürzlich (HEINEN u. LINSKENS 1960, HEINEN 1960) das Vorhandensein eines derartigen Enzyms in *Penicillium spinulosum* eindeutig nachgewiesen werden. Das Enzym, das die vorläufige Bezeichnung "Cutinase" erhielt, läßt sich mit Phosphatpuffer aus dem Mycel extrahieren, es kann mit Ammonsulfat ausgefällt werden und erwies sich als beständig gegen kurze Dialyse. Die Cutinase wurde bisher nur indirekt, nämlich durch die Bestimmung der Dehydrierung beim weiteren Abbau der durch das Primärenzym freigesetzten Cutin-Fettsäuren nachgewiesen. Die Beobachtung, daß durch die Einwirkung der Pilzenzyme das Cutin in verdünntem Alkali teilweise löslich wurde (HEINEN 1960) bot jedoch eine Möglichkeit zur direkten Verfolgung des Cutin-Abbaus. Weitere Untersuchungen in dieser Richtung ergaben, daß die durch die enzymatische Cutinolyse entstehenden freien Fettsäuren im umgebenden wässrigen Medium der Versuchsansätze nicht in Lösung gehen, daß jedoch bei anschließender Einwirkung verdünnten Alkalis die freien Säuregruppen mit der verwendeten Lauge reagieren. Bei Verwendung einer Kali- oder Natronlauge bekannter Normalität ergibt die Titration der nicht verbrauchten Lauge mit HCl die Menge an freien Fettsäuren, die unter Seifenbildung mit dem Alkali reagiert haben. Die aus einer bestimmten Menge Cutin unter verschiedenen Bedingungen freigesetzte Fettsäuremenge gibt wiederum Aufschluß über die Aktivität der verwendeten Enzymlösung. In der vorliegenden Arbeit wird einerseits die Cutinolyse unter der Einwirkung von Extrakten aus *P. spinulosum* mittels der Titrationsmethode durch Bestimmung der freigesetzten Fettsäuren verfolgt, andererseits werden auf diese Weise die optimalen Bedingungen für die Wirkung der Cutinase sowie einige Eigenschaften des Enzyms untersucht.

II. METHODIK

1. *Pilzmaterial und Züchtung*

Zu den Versuchen wurde der von rottenden Blättern isolierte Schimmelpilz *Penicillium spinulosum* Thom (früher als *P. spec.* bezeichnet) verwendet (RAPER u. THOM 1949). — Die Anzüchtung des Pilzes zu den Versuchen geschah wie früher (HEINEN 1960) angegeben.

2. *Herstellung der Enzymlösung*

Die Vorextraktion des trocken gepreßten Mycels mit Wasser und die Hauptextraktion mit Phosphatpuffer wurde wie früher angeben durchgeführt. Eine Vorreinigung der zum Rohextrakt "R" vereinigten beiden Extrakte erfolgte durch Zusatz von Protaminsulfat (2 mg pro ml Lösung) nach den Angaben von SCHLOSSMANN u. LYNEN (1957). Diese Enzymlösung wird mit "E" bezeichnet. Ammonsulfatfällungen wurden durch Zusatz festen Ammonsulfats zur Lösung R oder E unter gleichzeitigem Rühren bis zum Erreichen des gewünschten Sättigungsgrades hergestellt. Die ausgefällten Eiweiße wurden nach dem Abzentrifugieren in Phosphatpuffer pH 6,0, seltener in Wasser, suspendiert; nach 1-stündigem Stehen bei $+5°$ wurden alle unlöslichen Eiweiße abzentrifugiert und die so entstandenen, mit "F" bezeichneten klaren Lösungen, zu den Versuchen verwendet.

3. *Dialyse*

Zur "Normal-Dialyse" wurden die Enzymlösungen in einen unten verschlossenen Cellophanschlauch von 2,2 cm ⌀ gefüllt und bei +5° verschieden lange gegen dest. Wasser dialysiert. Zur "Intensiv-Dialyse" (HEINEN 1958) wurde in die im Cellophanschlauch befindliche Lösung ein mit Wasser gefülltes verschlossenes Reagensglas bis zum Boden eingetaucht und wiederum gegen dest. Wasser bei +5° dialysiert. Auf diese Weise wird die Oberfläche des Dialysiergutes stark vergrößert, die Dialyse verläuft dadurch einerseits rascher, andererseits ist durch die kürzeren Dialysierzeiten eine mögliche Schädigung der Enzymeiweiße zu vermeiden.

4. *Versuchsansätze*

Zur Mehrzahl der Versuche wurden je 10 mg Cutin in 10 ml der zu prüfenden Enzymlösung (R, E oder F) suspendiert und 14 Stdn bei 30° C in abgedeckten Bechergläsern oder verschlossenen Erlenmeyern inkubiert. Bei jeder Versuchsserie lief ein Kontrollansatz mit 10 mg Cutin in 10 ml Phosphatpuffer pH 6,0 mit. Veränderungen der Versuchsbedingungen hinsichtlich der Versuchszeit, der Temperatur, der Menge der Enzymlösung, oder andere Variationen sind jeweils im Text vermerkt.

5. *Bestimmung der freigesetzten Cutin-Fettsäuren*

a) Prinzip: Natives Cutin ist in heißem, konzentriertem Alkali unlöslich und kann nur durch Einwirkung von siedender, alkoholischer KOH oder durch Kochen am Rückflußkühler mit K_2CO_3 in Alkohol hydrolysiert und in Lösung gebracht werden. Hingegen reagieren die freien Fettsäuren aus Cutin direkt mit verdünnten Alkalien unter Bildung der entsprechenden Seifen. Sind durch die Wirkung von Enzymlösungen entsprechender Aktivität Fettsäuren aus dem Cutin abgespalten worden, so gehen diese im umgebenden wässrigen Milieu der Versuchsansätze nicht in Lösung. Überführt man derart vorbehandeltes Cutin in eine Alkalilösung bekannter Normalität, so wird bei der durch Erhitzen beschleunigten Seifenbildung eine bestimmte Menge der Lauge verbraucht, die der Menge freier Carboxylgruppen und somit der Menge Fettsäure äquivalent ist. Titriert man nun nach erfolgter Verseifung mit einer Säure gleicher Normalität, so wird umso weniger Säure verbraucht, je mehr Lauge bereits durch die Reaktion mit den Fettsäuren neutralisiert wurde. Zum Vergleich läuft jeweils einerseits der Kontrollansatz mit dem Cutin das in Pufferlösung angesetzt war, mit; andererseits ein Ansatz mit Lauge ohne Cutin, zur Kontrolle des Titers. Die Differenz zwischen dem Verbrauch an KOH durch das Cutin aus dem Kontrollansatz und dem aus der Enzymlösung ergibt die Menge der durch die Cutinase freigesetzten Fettsäuren.

b) Durchführung der Bestimmung: Das Cutin aus dem Kontrollansatz mit Pufferlösung und dem Versuchsansatz mit Enzymlösung wird aus der Lösung abfiltriert, gewaschen und getrocknet. Das Cutinpulver wird dann in trockene Reagensgläser überführt und das Gewicht bestimmt. Nach Zusatz von je 5,0 ml 0,01 n KOH werden die Gläser mit einer passenden hohlen Glaskugel lose verschlossen und in ein siedendes Wasserbad eingehängt. Nach 20 Min werden die Ansätze herausgenommen, abgekühlt und mit 0,01 n HCl unter Verwendung von Phenolphthalein als Indikator titriert. Der Verbrauch an HCl ergibt die durch die Verseifung neutralisierte Menge KOH, woraus die Menge der freigesetzten Fettsäure (ebenfalls in ml 0,01 n Lösung ausgedrückt) berechnet wird. Um bei Enzymlösungen mit unterschiedlichem Trockengewicht vergleichbare Werte zu erhalten, werden die Ergebnisse auf 1 mg Trockengewicht bezogen.

6. *Präparate*

Sowohl das zumeist verwendete Cutin von Gasteria verricuosa, als auch die Cutine von Aloe arborescens, Prunus domestica, Pyrus communis, Vitis vinifera und Solanum lycopersicum wurden nach der früher angegebenen Methode (ORGELL 1955, HEINEN 1960) hergestellt. Besonderheiten sind im Text angegeben.

III. Ergebnisse

1. *Nachweis der Cutinspaltung durch titrimetrische Bestimmung der Reaktionsprodukte*

Zur Orientierung über die zu erwartende Menge freier Fettsäuren die durch enzymatische Lyse des Cutins durch Einwirkung der Fermente aus *P. spinulosum* gebildet werden, wurden zunächst folgende Versuche angesetzt: Fein gemahlenes Cutin wurde 15 Stdn bei 30° C mit a) einer Mycelsuspension von *P. spinulosum* in Phosphatpuffer pH 7,2 b) einem Wasserextrakt aus dem trocken gepreßten Pilzmycel c) einem Pufferextrakt d) einer 1 : 1-Kombination beider Extrakte und e) einem hitze-inaktivierten (5 Min, 95° C) Pufferextrakt als Kontrolle inkubiert. Anschließend wurde der Verbrauch des Cutins an 0,01 n KOH durch Rücktitration mit 0,01 n HCl, also die Menge der entstandenen freien Fettsäuren (ausgedrückt in ml 0,01 n Fettsäure, vgl. Methodik), bestimmt. Bei einer Substratkonzentration von je 20 mg Cutin pro Ansatz ergab sich, daß durch

a) Mycelsuspension:	$0{,}18 \times 10^{-3}$ ml	0,01 n	Fettsäuren/10 mg	Cutin/Tr.	G.
b) Wasserextrakt :	$1{,}72 \times 10^{-3}$ ml		„	„	„
c) Pufferextrakt :	$0{,}67 \times 10^{-3}$ ml		„	„	„
d) Kombination :	$1{,}82 \times 10^{-3}$ ml		„	„	„
e) Extrakt, inaktiv.:	$0{,}04 \times 10^{-4}$ ml		„	„	„

freigesetzt worden waren. Wenn es sich dabei auch absolut gesehen um sehr geringe Mengen handelt, so reichen diese doch aus, um die Bildung der freien Fettsäuren aus dem Substrat unter Einwirkung der Cutinase aus *P. spinulosum* zu verfolgen und relative Unterschiede durch Variation der Versuchsbedingungen bestimmen zu können. Obwohl die Aktivität des Pufferextraktes geringer scheint als die des Wasserextraktes, wurde doch aufgrund der früheren Befunde (Heinen u. Linskens 1960, Heinen 1960) zu den weiteren Untersuchungen ein Rohextrakt (R) verwendet, der durch Vorextraktion des Mycels mit Wasser und anschließender Extraktion mit Phosphatpuffer und Vereinigung beider Extrakte erhalten wurde.

a) *Versuche zur Ausschaltung störender Faktoren*

Die einleitenden Versuche zur Festlegung der günstigsten Versuchsbedingungen in Bezug auf die Art des Ansatzes, die Menge des zu verwendenden Extraktvolumens und die Versuchszeit, wurden mit dem Rohextrakt (R) aus *P. spinulosum* durchgeführt. Ansätze mit 20 ml Rohextrakt und 12 mg Cutin pro Ansatz wurden zur ersten Orientierung einerseits im Thermostaten bei 30° C, andererseits auf der Schüttelmaschine bei 27,5° C inkubiert. Die anschließende Titration der freigesetzten Fettsäuern nach 22 Stdn ergab, daß im geschüttelten Ansatz rund 75 % weniger an Reaktionsprodukten vorlag als in den nicht geschüttelten im Thermostaten bewahrten Proben, weshalb für die folgenden Versuche keine Schüttelansätze benutzt wurden.

Bei Versuchen mit einerseits unterschiedlichen Volumina Roh-

extrakt, andererseits verschieden langen Inkubationszeiten, ergab sich der unerwartete Befund, daß sowohl die Erhöhung der Enzymlösungsmenge als auch die Verlängerung der Inkubationszeit einen geringeren Anfall freigesetzter Fettsäure zur Folge hat (Tab. I). Die Ergebnisse lassen sich folgendermaßen interpretieren:

Tabelle I

Freisetzung von Fettsäuren aus Cutin durch Rohextrakt aus *P. spinulosum* bei Variation der Versuchszeit oder des Ansatzvolumens.

Versuchs-zeit	ml R pro Ansatz	ml 0,01 n freigesetzte Fettsäure
12 Stdn		$2{,}37 \times 10^{-3}$
24 ,,	12	$1{,}94 \times 10^{-3}$
48 ,,		$0{,}62 \times 10^{-3}$
	20,0	$0{,}90 \times 10^{-4}$
	15,0	$0{,}58 \times 10^{-3}$
24 Stdn	10,0	$1{,}14 \times 10^{-3}$
	5,0	$1{,}10 \times 10^{-3}$
	2,5	$0{,}13 \times 10^{-3}$

Im Extrakt befinden sich neben anderen auch die Enzyme des Fettsäureabbaus. Hierzu gehören die in Schimmelpilzen weit verbreiteten Enzyme der Methylketonbildung (Stärkle 1924, Stokoe 1928, Thaler u. Geist 1939, Thaler u. Stählin 1949, Franke u. Heinen 1958 a), wozu die in *Penicillium-* (Karrer u. Haab 1948) und *Aspergillus*-Arten (Franke, Heinen u. Platzeck 1959) nachgewiesene ß-Ketosäuren-decarboxylase zählt. Vorhanden sind außerdem Fettsäure-Dehydrasen (Mukherjee 1951, 1952, Franke u. Heinen 1958 b, Heinen u. Linskens 1960, Heinen 1960), die im Gegensatz zu pflanzlichen Fettsäure-Dehydrasen (vgl. Übersicht bei Franke u. Frehse 1957) CoA-abhängig sind und durch Dialyse inaktiviert werden, so wie die übrigen, nach der Fettsäureabbauspirale von Lynen (1955) am Fettsäurestoffwechsel beteiligten Enzyme. Durch das Zusammenwirken dieser Fermente werden die durch die Cutinase freigesetzten Fettsäuren weiter umgesetzt und somit dem hier angewandten Titrationsnachweis entzogen. Die Bestätigung für die Richtigkeit dieser Annahme ergab sich aus Versuchen zum Verhalten der Extrakte gegenüber Dialyse. Vorversuche zeigten bereits, daß durch Normaldialyse eine scheinbare Steigerung der Cutinase-Aktivität eintrat. Zur genaueren Verfolgung der Dialyse-Wirkung wurde der Rohextrakt einer "Intensivdialyse" bei $+5°$ C gegen dest. Wasser unterzogen und die Aktivität der Lösung nach 4, 6, 14 und 24 Stdn geprüft. Wie Fig. 1 zeigt, lassen sich mit steigender Dialysierzeit bis zu einem Optimum nach etwa 10 Std. stets mehr freigesetzte Fettsäuren nachweisen. Die scheinbare Steigerung der Cutinase-Aktivität beruht dabei de facto auf einer fortschreitenden Inaktivierung der Begleitfermente, deren Ausfall zu einer Anhäufung der Cutin-Spaltprodukte führt, die einem weiteren Umsatz nicht mehr ausgesetzt sind. Der höhere Gehalt des Pufferextraktes an Abbau-Enzymen

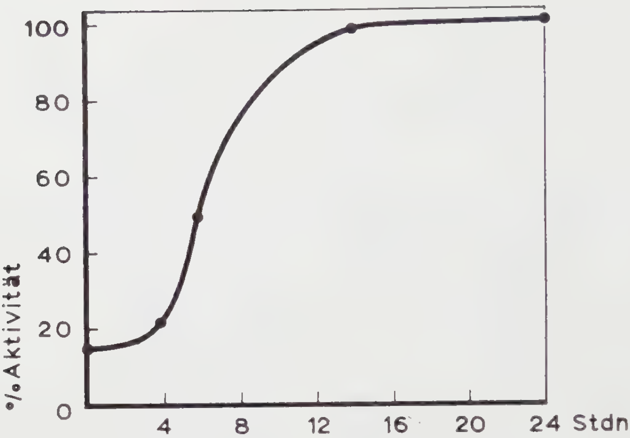

Fig. 1. Aktivität der Cutinase (ausgedrückt in %-Aktivität, wobei der Höchstwert von 2,45 × 10^{-3} ml 0,01 m Fettsäure = 100 gesetzt ist) nach verschieden langer Intensivdialyse bei +5° gegen dest. Wasser. Titration der aus Cutin gelösten Fettsäuren nach 12-stündiger Inkubation.

erklärt auch die im Widerspruch zu früheren Ergebnissen (HEINEN 1960) stehende geringere Cutinase-Aktivität gegenüber dem Wasserextrakt: Nach 8-stündiger Intensivdialyse steigt die Aktivität bereits auf den 3-fachen Wert, die Aktivität des Wasserextraktes nimmt in der gleichen Zeit nur um 1/6 des Ausgangswertes zu.

Die Verminderung der nachweisbaren Fettsäuremenge bei Erhöhung des Extraktvolumens pro Ansatz läßt sich auf die gleiche Weise erklären: Zugleich mit der Cutinase-Konzentration nimmt auch die Konzentration der Abbau-Enzyme zu, wobei das Gleichgewicht mit der Volumenzunahme derart verschoben wird, daß ein stets intensiverer Umsatz der freiwerdenden Fettsäure einsetzt, da die Konzentration bestimmter Abbau-Enzyme, wie beispielsweise der Dehydrase im Mycel recht gering ist (FRANKE u. HEINEN 1958 b), so daß diese erst von einem bestimmten optimalen Volumen an ihre volle Wirkung entfalten.

b) *Protaminsulfat- und Ammoniumsulfat-Fällungen aus Rohextrakten*

Aufgrund der Ergebnisse der Dialyseversuche schien es angebracht, im Folgenden zunächst zu versuchen, die Rohextrakte von störenden Begleitfermenten zu säubern, was natürlich durch Dialysieren erreicht werden kann, besser jedoch mittels Trennung der aktiven und inaktiven Eiweiße durch Fällungsmethoden. Nachdem bereits früher festgestellt worden war, daß sich die Cutinase mit Ammonsulfat ausfällen läßt (HEINEN 1960), ergaben weitere Versuche dieser Art, daß das gesamte in Bezug auf die Cutinspaltung aktive Eiweiß bei 60–70 %iger Sättigung der Rohextrakte mit Ammonsulfat ausgefällt werden kann (Tab. II); bei niedrigeren Sättigungsgraden ist die

TABELLE II

Aktivität der Cutinase nach Ausfällung bei verschiedenen Ammonsulfatsättigungsgraden aus Rohlösung: Fettsäurebildung aus Cutin durch 24-stündige Inkubation mit den Lösungen der einzelnen Fällungen.

Ammonsulfat-Konzentration	Trockengewicht in mg/ml [1])	ml 0,01 n Fettsäure
0 % = Rohlösg.	9,33	$0{,}98 \times 10^{-3}$
50 %	6,97	$0{,}91 \times 10^{-3}$
60 %	7,44	$1{,}21 \times 10^{-3}$
70 %	7,72	$1{,}23 \times 10^{-3}$
85 %	9,15	$1{,}04 \times 10^{-3}$

[1]) Alle Ansätze mit 12,0 ml Lösung.

Ausfällung offenbar nicht vollständig, bei Konzentrationen über 70 % fallen dagegen weitere inaktive Eiweiße aus. Aktivitätssteigerung konnte auf diese Weise jedoch nicht erzielt werden, was z.T. darauf zurückzuführen ist, daß der Eiweißgehalt und somit das Trockengewicht der Lösung der Ammonsulfatfällung (= F) im Vergleich zur Ausgangslösung nur wenig vermindert ist. Bessere Ergebnisse brachte hingegen die Behandlung der Rohlösung mit Protaminsulfat (2 mg/ml Lösung) zur Entfernung der Nucleinsäuren nach SCHLOSSMANN u. LYNEN (1957): Die Aktivität der nucleinsäure-freien Enzymlösung (E) liegt etwa 3–4 mal höher als in der Ausgangslösung (vgl. Fig. 2).

Als brauchbare Methode ergab sich schließlich die Kombination beider Verfahren, nämlich eine Vorreinigung des Rohextraktes durch Fällung der Nucleinsäuren mit Protaminsulfat und anschliessende

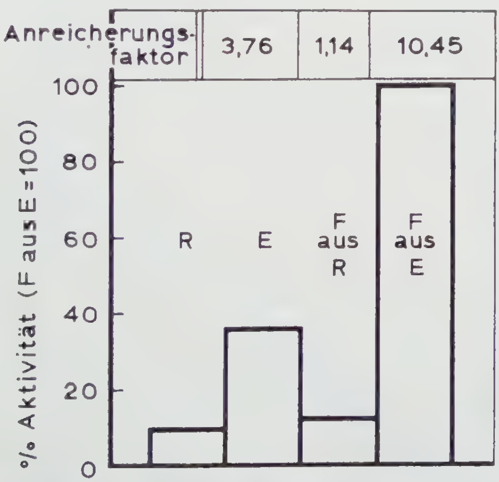

Fig. 2. Anreicherung der Cutinase durch Fällung bei 70 %iger Ammonsulfatkonzentration aus Rohextrakt (R) und aus durch Protaminsulfatbehandlung vorgereinigtem Extrakt (E).

Bestimmung der Cutin-Lyse nach 10-stündiger Inkubation der einzelnen Enzymlösungen bei 30° C mit je 10 mg Cutin pro Ansatz.

Fällung bei 70 %iger Sättigung der Lösung E mit Ammonsulfat und Aufnehmen der Fällung in Phosphatpuffer von pH 6,0, wobei nach Abzentrifugieren der unlöslichen Eiweiße eine klare Lösung (F) entsteht (Fig. 2). Interessant war die Beobachtung, daß eine fast gleich starke Anreicherung auch erzielt wird, wenn statt der Protaminsulfatfällung eine 10-stündige Normaldialyse (oder 6-stündige Intensivdialyse) der Ammonsulfatfällung vorausgeht (Tab. III). Zu den

TABELLE III

Fällung der Cutinase bei 70 %iger Ammonsulfatsättigung aus nicht dialysiertem und dialysiertem Rohextrakt. Bestimmung der freigesetzten Fettsäuren nach 10-stündiger Inkubation mit je 10 ml Enzymlösung.

Enzymlösung	ml 0,01 n freigesetzte Fettsäure	Anreicherung
R	$0{,}35 \times 10^{-3}$	–
R, 10 h dialys. . .	$1{,}18 \times 10^{-3}$	3,38 – fach
F (70 %) aus R . .	$0{,}44 \times 10^{-3}$	1,26 – fach
F aus R (dialys) .	$3{,}42 \times 10^{-3}$	9,79 – fach

weiteren Versuchen wurden, wenn nicht anders angegeben, stets Enzymlösungen benutzt, die auf die oben angegebene Weise durch Extraktion, Vorreinigung der Rohlösung mit Protaminsulfat und anschließende Fällung bei 70 %iger Ammonsulfatsättigung erhalten worden waren. Zwar wird dabei eine nur etwa 10-fache Anreicherung des Enzyms erzielt, doch enthalten die Lösungen keine aktiven cofaktor-abhängigen Enzyme des Fettsäureabbaus mehr, was zur Erfassung der durch die Cutinspaltung entstandenen Fettsäuren und somit zur Beurteilung der Aktivität der Cutinase unter variierten Bedingungen wesentlich ist.

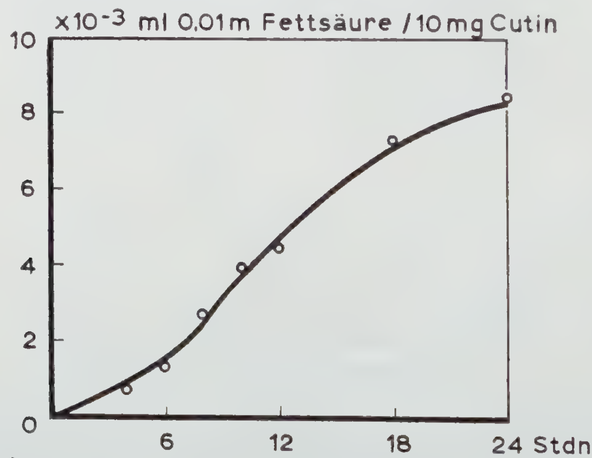

Fig. 3. Abhängigkeit der Cutinolyse von der Einwirkungszeit der Cutinaselösung, ausgedrückt in ml gebildeter Fettsäure (0,01 m).

2. *Zur Kinetik der Cutinase aus* P. spinulosum

a) Zur Beziehung zwischen Versuchszeit und der Cutinspaltung

Die Menge an freigesetzten Fettsäuren, die durch Einwirkung der Enzymlösung "F" auf *Gasteria*-Cutin entsteht, wurde mehrfach nach verschieden langer Einwirkungsdauer bestimmt. Die Auswertung dieser Versuchsreihen ergab, daß die Spaltung des Cutins innerhalb der ersten sechs Stunden langsam einsetzt, zwischen sechs und vierzehn Stunden stärker wird und danach, wahrscheinlich infolge einer Hemmung des Enzyms durch die Anhäufung von Reaktionsprodukten, langsam an Intensität verliert (Fig. 3). Bei allen weiteren Versuchen wurde deshalb mit konstanter Versuchszeit gearbeitet, oder die veränderte Versuchsdauer entsprechend berücksichtigt.

b) Substratkonzentration

Der Einfluß der Substratkonzentration auf die enzymatische Cutinspaltung wurde durch Inkubation steigender Mengen fein gemahlenen Cutins mit konstantem Volumen (10 ml) Enzymlösung untersucht. Es ergab sich, daß optimale Cutinolyse in Anwesenheit von 1,0–1,5 mg Substrat pro ml Enzymlösung erfolgt (Fig. 4). Die graphische Ermittlung der Michaelis-Konstanten unter Zugrundelegung eines Molekulargewichtes von 300 betrug $1{,}7 \times 10^{-3}$ m.

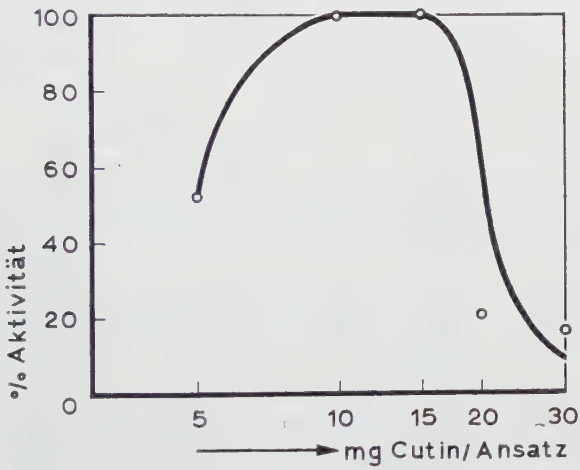

Fig. 4. Einfluß der Substratkonzentration (mg Cutin/10 ml Enzymlösung) auf die Aktivität der Cutinase.

c) Abhängigkeit der Aktivität von der Versuchstemperatur

Unter Beachtung der optimalen Substratkonzentration und Einwirkungszeit wurden Ansätze mit konstantem Volumen an Enzymlösung bei 2, 8, 15, 22, 30, und 40° inkubiert. Die Bestimmung der freigesetzten Fettsäuren nach Versuchsende ergab, daß die Cutin-

spaltung in einem Bereich von 15–35° C unabhängig von der Temperaturerhöhung verläuft (Tab. IV). Unterhalb 15° C sinkt die Aktivität allerdings sehr rasch ab, der Aktivitätsverlust oberhalb des Optimalbereiches ist auf eine beginnende Schädigung (vgl. Temperaturstabilität!) des Proteins zurückzuführen.

Tabelle IV

Einfluß der Inkubationstemperatur auf die Cutinspaltung durch eine Enzymlösung aus *P. spinulosum*.

Versuchstemperatur	2	8	15	22	30	40	°C
ml Fettsäure aus 10 mg Gasteriacutin	2,75	7,60	11,90	12,55	12,60	11,21	x 10^{-3}

d) pH-Aktivität der Cutinase

Die pH-Aktivität des Enzyms wurde für den Bereich von pH 4 bis pH 9,5 dadurch ermittelt, daß Ansätze unter sonst konstanten Bedingungen unter Variation des pH-Wertes durch die Verwendung entsprechender Puffer inkubiert wurden. Um pH-Verschiebungen durch den Puffer der Enzymlösung zu vermeiden, wurden die mit Ammonsulfat ausgefällten Eiweiße in Wasser suspendiert und die daraus erhaltenen wässrigen F-Lösungen verwendet. Wie aus Fig. 5 zu ersehen ist, hat das Ferment ein ausgeprägtes Optimum bei pH 6,0 von dem aus die Aktivität zur sauren Seite hin bedeutend steiler abfällt als zur alkalischen Seite.

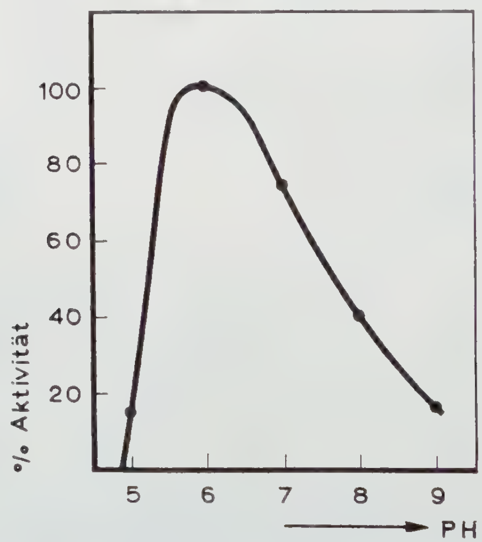

Fig. 5. Aktivität des cutinspaltenden Enzyms aus *P. spinulosum*-Extrakt in Abhängigkeit vom pH-Wert des Inkubationsansatzes.

e) Enzymkonzentration

Die Bestimmung der Cutinspaltung in Ansätzen mit steigendem Gehalt an Enzym ergab, wie Fig. 6 zeigt, einen Bereich der Proportionalität zwischen der Enzymkonzentration und der katalysierten Reaktion der nach unten hin mit 7,5 mg Enzymtrockengewicht, nach oben durch den Wert 22,5 mg Enzymtrockengewicht pro Ansatz begrenzt wird. Hieraus ließ sich ermitteln, daß alle bisherigen Versuche mit der angereicherten Lösung F im Bereich der optimalen

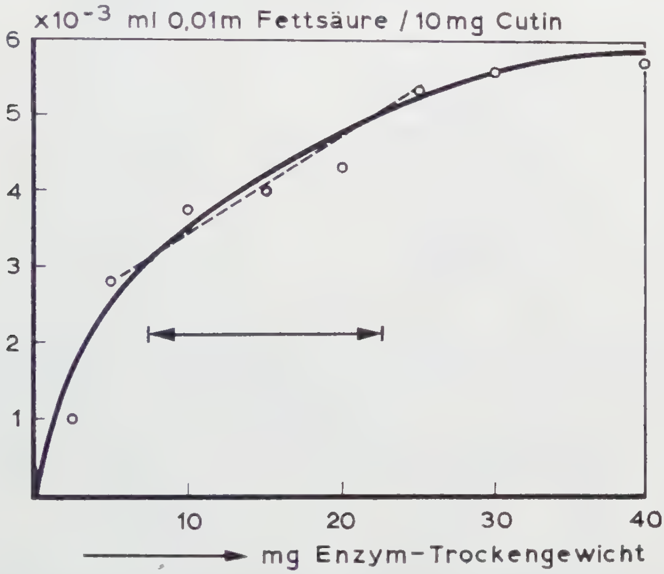

Fig. 6. Beziehungen zwischen der Enzymkonzentration (mg Enzymtrockengewicht/ml Lösung) und der Intensität der Cutinspaltung (ml 0,01 m Fettsäure gebildet pro 10 mg Cutin; ↔ = Proportionalitätsbereich).

Enzymkonzentration durchgeführt waren, und bei allen weiteren Untersuchungen wurde darauf geachtet, daß die Grenzen des Bereichs eingehalten wurden.

3. Versuche zur Substratspezifität des Enzyms

Zu den folgenden Versuchen wurden Cutine verschiedener Herkunft benutzt, die jedoch alle nach der früher angegebenen Methode isoliert wurden. Verwendet wurden zwei Blatt-Cutine, nämlich einerseits Cutin von den Blättern der *Gasteria verricuosa*, mit dem alle bisherigen Versuche durchgeführt wurden und andererseits Cutin von *Aloe arborescens*. Daneben wurden vier Frucht-Cutine isoliert und zwar von *Prunus domestica*, *Pyrus communis*, *Solanum lycopersicum* und *Vitis vinifera*. Außerdem wurden Griffel-, bzw. Narben-Cutine von *Petunia hybrida* und *Brassica nigra* als Substrate verwendet. Bei *Petunia* wurde etwa 1/5 des oberen Stempelteils abgeschnitten, bei *Brassica* die Narbe

vom Griffel getrennt und der Pectinaseeinwirkung ausgesetzt, worauf eine Nachbehandlung mit 96 %igem Alkohol folgte.

Die Verwendung der verschiedenen Cutine als Substrat ergab, daß die beiden Blatt-Cutine mit etwa gleicher Intentsität angegriffen werden (Tab. V). Hingegen ist bei den Cutinen der Fruchtschalen eine höhere Abbau-Aktivität gegeben, die von den beiden Stempel-

Tabelle V

Lyse von Cutin verschiedener Herkunft durch *Penicillium spinulosum* Extrakt.

Cutin isoliert von	ml 0,01 n Fettsäure aus 10 mg Substrat
Gasteria verricuosa . . .	$3{,}4 \times 10^{-3}$
Aloe arborescens	$4{,}0 \times 10^{-3}$
Prunus domestica	$14{,}0 \times 10^{-3}$
Pyrus communis	$17{,}4 \times 10^{-3}$
Solanum lycopersicum . .	$8{,}2 \times 10^{-3}$
Vitis vinifera	$8{,}4 \times 10^{-3}$
Brassica nigra	$5{,}2 \times 10^{-2}$
Petunia hybrida	$3{,}5 \times 10^{-2}$

cutinen jedoch noch weit übertroffen wird. Die Befunde legen die Schlußfolgerung nahe, daß die Differenzen auf Unterschiede im Aufbau der hochpolymeren, vernetzten Substrate zurückzuführen sind. Außer strukturellen Unterschieden können auch chemische Verschiedenheiten hinsichtlich der Kettenlänge und der Mengenverhältnisse der einzelnen Fettsäuren für das unterschiedliche Verhalten der Substrate gegenüber dem Enzym verantwortlich sein.

Weiterhin wurde in diesem Zusammenhang untersucht, wie sich der Zustand des Substrates auf die Enzymaktivität auswirkt. Dazu wurde einerseits frisches *Gasteria*-Cutin als Substrat verwendet, andererseits wurde aber auch das Cutin aus den Versuchsansätzen wieder

Tabelle VI

Aktivität einer Enzymlösung aus *P. spinulosum* gegenüber Cutin, das mehrfach als Substrat verwendet wurde (vgl. Text).

Substrat-Alter	Enzym-Aktivität
Gasteria-Cutin, frisch isoliert.	74,35 %
Cutin, 2. Verwendung als Substrat	100,00 %
Cutin, 3. Verwendung als Substrat	53,82 %
Unverseifbarer Rest nach Alkalihydrolyse . .	0,02 %

isoliert, z.T. ein zweites Mal benutzt und wieder isoliert. So standen neben dem Frischmaterial zwei ältere Substrate zur Verfügung, die zum 2. resp. 3. Mal der Enzymwirkung ausgesetzt werden konnten. Außerdem wurde noch der bei der Hydrolyse von *Gasteria*-Cutin mit alkoholischer KOH verbleibende "unverseifbare Rest" als Substrat angeboten. Wie Tab. VI zeigt, ist die Fettsäurebildung bei dem

Cutin, das bereits einmal als Substrat gedient hat, stärker als beim Frischmaterial. Das kann u.U. dahingehend erklärt werden, daß durch die Ersteinwirkung durch Herauslösen eines Teils der Fettsäuren eine vergrößerte Oberfläche entstanden ist, die dem Enzym dann eine bessere Angriffsfläche bietet. Der rasche Abfall der Aktivität bei weiterer Verwendung steht wohl damit im Zusammenhang, daß das angreifbare Material stark vermindert ist. Aus dem "Unverseifbaren" werden praktisch keine Fettsäuren freigesetzt.

4. *Untersuchungen über die Stabilität des Enzyms*

a) pH-Stabilität

Die Widerstandsfähigkeit des Enzyms gegen verschiedene H-Ionenkonzentrationen wurde geprüft, indem die Ammonsulfatfällungen in der Hälfte des üblichen Volumens in Wasser gelöst und mit Puffer des gewünschten pH-Wertes zum Normalvolumen aufgefüllt wurden. Diese Lösungen wurden ohne Substrat 30 Min bei 30° C inkubiert, dann anschließend bei normalem Versuchs-pH (6,0) mit Substrat in der üblichen Weise angesetzt. Der optimale Stabilitätsbereich liegt zwischen pH 8,5 bis 9,5 (Fig. 7), der Abfall der Aktivität zur linken Seite hin erfolgt langsam, während das Enzym gegenüber pH-Werten oberhalb 10 sehr empfindlich ist. Unterhalb pH 5 wurden Eiweißausflockungen bei den Versuchen beobachtet, wonach cutinolytische Aktivität nicht mehr festzustellen war.

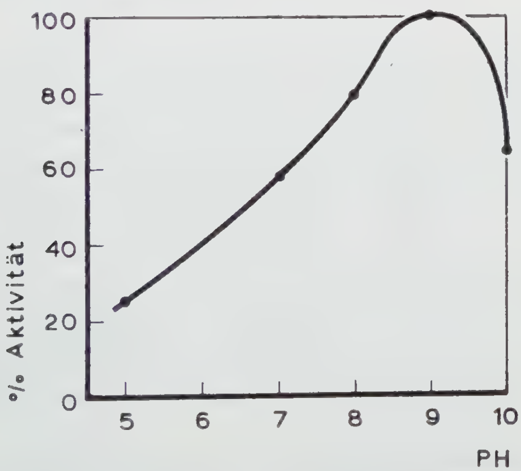

Fig. 7. pH-Einfluß auf die Stabilität des cutinspaltenden Enzyms aus *P. spinulosum* nach halbstündiger Inkubation bei bestimmten pH-Werten und anschließendem Ansatz mit Substrat bei pH 6,0.

b) Temperatur-Stabilität

Sie wurde durch 30 Min langes Erwärmen der Enzymlösung (je 2×10 ml) auf resp. 20, 30, 40, 45, 50, 55 und 60° C ermittelt. Die Lösungen wurden dann mit Substrat bei der normalen Versuchs-

temperatur angesetzt und schließlich die Aktivität der einzelnen Proben bestimmt. Die graphische Ermittlung der "Tötungstemperatur" (Fig. 8) nach der Definition von v. EULER (1925) ergab einen Wert von 57° C.

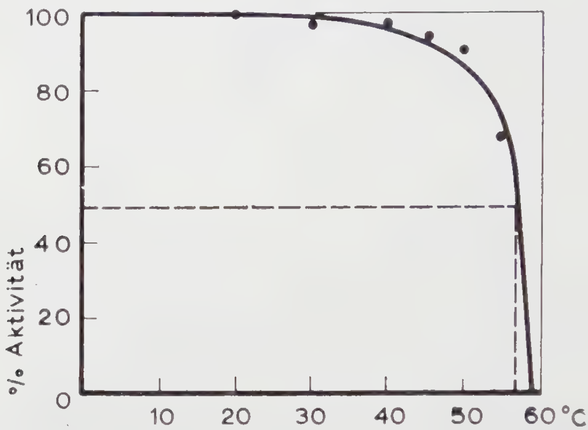

Fig. 8. Thermolabilität der Cutinase: Cutinspaltung nach halbstündiger Einwirkung von Temperaturen von 0–50° C auf eine Enzymlösung aus *P. spinulosum.*

c) Schüttelversuche (mechanische Stabilität)

Versuche zur Verfolgung des Cutinabbaus in Ansätzen, die auf der Schüttelmaschine bei Raumtemperatur durchgeführt wurden, ergaben eine bedeutend geringere Fettsäurebildung als in denjenigen Ansätzen, die im Brutschrank bei 30° C stehend inkubiert wurden. Eine genauere Verfolgung dieser Frage wurde in Versuchen durchgeführt in denen sowohl ein Rohextrakt R als auch eine angereicherte Enzymlösung F einerseits im Brutschrank bei 30° C, andererseits unter intensivem Schütteln auf der Schüttelmaschine inkubiert wurden. Wie aus den Werten der Tab. VII zu ersehen ist, liegt die Aktivität des Enzyms bei beiden Extrakten durch das Schütteln wesentlich niedriger im Vergleich zu den anderen Ansätzen. Daraus kann auf eine "Schüttel-Inaktivierung" wie sie ähnlich auch bei anderen Enzymen, z.B. der Lipoxydase (FRANKE u. FREHSE 1953), schon beobachtet wurde, geschlossen werden.

TABELLE VII

Teilinaktivierung des cutinolytischen Enzyms aus *P. spinulosum* durch Schütteln der Versuchsansätze bei 25° C.

Versuchsansatz	Freigesetzte Fettsäure (0,01 m)
R bei 30°, *nicht* geschüttelt .	$1{,}71 \times 10^{-3}$ ml
R bei 25°, *geschüttelt*	$0{,}42 \times 10^{-3}$ ml
F bei 30°, *nicht* geschüttelt .	$11{,}45 \times 10^{-3}$ ml
F bei 25°, *geschüttelt*	$3{,}96 \times 10^{-3}$ ml

d) Alterungs- und Dialyse-Versuche

Im Laufe der Versuche hatte sich die Cutinase bereits als ein recht stabiles Ferment erwiesen. Zur Feststellung, ob das Alter der Extrakte einen Einfluß auf die Aktivität des Enzyms hat, wurden angereicherte F-Lösungen über längere Zeit (2, 5, und 10 Tage) im Kühlraum bei +5° C stehen gelassen und danach zu Ansätzen mit Substrat unter den üblichen Bedingungen verwendet. Die Angaben der Tab. VIII (oberer Teil) zeigen, daß nach 48-stündiger Alterung ein geringer Anstieg der Aktivität zu verzeichnen ist, daß die Aktivität dann über 3 weitere Tage konstant bleibt und nach 10 Tagen erst etwas abgesunken ist.

Tabelle VIII

Einfluß der Alterung und der Dialyse (Intensiv-Dialyse) auf die Enzym-Aktivität.

Enzymlösung	Titrationswert nach Verseifung
frisch	$9{,}4 \times 10^{-3}$ ml
2 Tage bei +5°	$10{,}3 \times 10^{-3}$ ml
5 Tage bei +5°	$10{,}1 \times 10^{-3}$ ml
10 Tage bei +5°	$9{,}7 \times 10^{-3}$ ml
frisch	$9{,}1 \times 10^{-3}$ ml
6 Stdn Intensivdialyse	$12{,}8 \times 10^{-3}$ ml
12 Stdn Intensivdialyse	$12{,}5 \times 10^{-3}$ ml
18 Stdn Intensivdialyse	$12{,}7 \times 10^{-3}$ ml
24 Stdn Intensivdialyse	$11{,}2 \times 10^{-3}$ ml

Aufgrund des Verhaltens der Rohextrakte bei der Dialyse (Fig. 1) wurde auch die Beständigkeit der angereicherten Lösung geprüft. Hierzu wurden je 10 ml Lösung resp. 6, 12, 18 und 24 Stdn gegen dest. Wasser bei +5° C dialysiert (Intensiv-Dialyse) und danach ihre Aktivität gegen Cutin mit der des frischen Extraktes verglichen. Aus Tab. VIII (unterer Teil) ergibt sich ein Anstieg der Aktivität nach 6-stündiger Intensiv-Dialyse, danach bleibt diese konstant und beginnt erst nach 24 Std. abzufallen, wahrscheinlich aufgrund einer Proteinschädigung. Der sowohl bei der Alterung als auch bei der Dialyse beobachtete Aktivitätsanstieg zu Beginn der Behandlung ist, analog zu den Ergebnissen früherer Versuche mit der Rohlösung R, auf eine Inaktivierung von noch in geringer Konzentration vorhandener dialyse-empfindlicher Begleitfermente zurückzuführen.

5. *Versuche zur allgemeinen Charakteristik des Enzyms*

a) Aktivitätsprüfungen unter verschiedener Gasatmosphäre

Die Frage, ob die Aktivität des Enzyms durch bestimmte Gase beeinflußt werden könnte, wurde in Versuchen geprüft, bei denen ein langsam perlender Gasstrom durch die Ansätze geleitet wurde. Luft wurde durch eine kleine Pumpe über ein Wattefilter in die Lösung geleitet, die anderen Gase, nämlich Sauerstoff, Stickstoff und Kohlen-

säure wurden aus Druckflaschen ebenfalls über Wattefilter eingeleitet. Die Versuche ergaben (Tab. IX) einen Anstieg der Aktivität gegenüber dem Normalversuch bei Einleitung von Sauerstoff oder Luft. Aber auch bei Verwendung von CO_2 oder N_2 lagen die Werte etwas höher als bei dem Vergleichsansatz. Daraus ergibt sich, daß das Enzym unter allen gebotenen Atmosphären aktiv ist. Die bei der Durchlüftung beobachtete Aktivierung ist wahrscheinlich nicht auf die Wirkung des einen oder anderen Gases zurückzuführen, sondern auf die bessere Verteilung des Substrates durch den durchperlenden Gasstrom.

Tabelle IX

Intensität der Cutinspaltung bei Begasung unter Sauerstoff-, Luft-, Kohlensäure- und Stickstoffatmosphäre im Vergleich zum normalen, nicht belüfteten Ansatz.

Versuchsansatz	Cutinase-Aktivität
normal bei 30° . .	81 %
belüftet mit O_2 . .	100 %
belüftet mit Luft .	98 %
belüftet mit CO_2 .	88 %
belüftet mit N_2 . .	84 %

b) Einige Bestimmungsversuche zum Enzymtyp

Nachdem aus den Belüftungsversuchen bereits deutlich geworden war, daß die Cutinase kein O_2-abhängiges Enzym ist, wurden in weiteren Versuchen verschiedene andere Enzyme im Extrakt bestimmt und ihr Verhalten bei der Anreicherung verfolgt. Dabei ergab sich, daß die Konzentration der im Rohextrakt nachweisbaren *Katalase* (Gagnon, Hunting u. Esselen 1959), *Esterase* (Stansly u. Ramsey 1956, Muftic 1954, Barnett u. Seligman 1951) und *Pectinase* (Orgell 1955) im angereicherten Extrakt F bedeutend geringer ist. Demnach ist die Cutinase mit keinem dieser Enzyme identisch. Angaben über den Wirkungsmechanismus des Enzyms sind aufgrund der bisherigen Ergebnisse nicht möglich.

c) Zur Lokalisation des Enzyms

Zur Prüfung der Frage, ob das im Mycelextrakt nachgewiesene cutinspaltende Enzym in den Mitochondrien oder im Cytoplasma der Zellen lokalisiert ist, wurde ein wie üblich gewonnener Rohextrakt R in der Kühlzentrifuge 25 min lang bei -3° und 22.000 g zentrifugiert. Die Mitochondrien, die sich bei dieser Behandlung als brauner Niederschlag abscheiden, wurden in Phosphatpuffer pH 6,0 aufgenommen und zwar im halben Volumen der Ausgangslösung. Der mitochondrien-freie Überstand wurde dann wie üblich mit Protaminsulfat und Ammonsulfat gefällt und die erhaltene Enzymlösung F' mit einer normalen Lösung F aus mitochondrienhaltigem Rohextrakt verglichen. Die Aktivitätsprüfung der drei Lösungen F, F' und M (= Mitochondriensuspension) ergab, daß die Mitochondrien keinerlei Aktivität enthalten, und daß durch vorheriges Auszentrifugieren der

Partikel die Aktivität der Enzymlösung F′ gegenüber F gesteigert werden kann (Tab. X).

Tabelle X.
Bestimmung der Lokalisation der Cutinase (vgl. Text).

Enzymlösung	ml 0,01 n freigesetzte Fettsäure
F aus mitochondrienhaltiger Lösung .	$7{,}1 \times 10^{-3}$
F′ aus mitochondrienfreier Lösung . .	$11{,}5 \times 10^{-3}$
Mitochondrien-Suspension "M" . . .	0

IV. Zusammenfassung

1) Das cutinspaltende Enzym in Extrakten aus *Penicillium spinulosum* läßt sich durch Vorreinigung mit Protaminsulfat und Ammonsulfatfällung bei 70 %iger Sättigung etwa 10-fach anreichern. Dabei können zugleich störende Begleitfermente abgetrennt werden.

2) Die Ermittlung optimaler Reaktionsbedingungen ergaben: Für die Substratkonzentration einen Bereich von 1,0–1,5 mg Cutin pro ml Enzymlösung, Unabhängigkeit der Reaktion von der Temperatur in einem Bereich von 15–35° C, optimales pH von 6,0 sowie einen Proportionalitätsbereich für die Enzymkonzentration von 7,5–22,5 mg Enzymtrockengewicht pro Ansatz.

3) Die Prüfung der Substratspezifität zeigte, daß außer dem Cutin von Blättern auch das von Früchten und Stempeln angegriffen wird.

4) Die Stabilität des Enzyms gegenüber der H-Ionenkonzentration, der Temperatur, Alterung und Dialyse wurde geprüft, wobei sich das Ferment als recht beständig und unabhängig von dialysablen Co-Faktoren erwies.

5) Weitere Versuche dienten zur allgemeinen Charakterisierung des Enzyms, dessen Wirkungsweise bisher nicht geklärt werden konnte. Die Cutinase ist kein Mitochondrienenzym, sondern im Cytoplasma der Zellen lokalisiert.

SUMMARY

1) A cutinolytic enzyme present in extracts of *P. spinulosum* was enriched by purification of the extract with protamine sulfate followed by treatment with ammonium sulfate at 70 % salt concentration. A separation from disturbing enzymes is obtained this way.

2) Determination of the optimum reaction conditions gave these results: Optimum of substrate concentration 1,0–1,5 mg cutin/ml enzyme solution, independence of the reaction from temperature between 15–35° C, pH-optimum at pH 6,0 and proportionallity between concentration of enzyme and destruction of substrate from 7,5 to 22,5 mg dry weight per ml sample.

3) Examination of substrate specifity with different cutins from leaves, fruits and stamps showed, that cutin isolated of stamps is much better attacked than all other materials tested.

4) The stability of the enzyme against H-ion concentration, temperature, aging and dialysis has been tested. According to the results obtained, cutinase is a stable enzyme needing no dialysable co-factors. Evidence has been made, that cutinase is inactivated by shaking.

5) Further examinations were done for general characterisation of the enzyme but failed in getting a definition about the enzymes' mechanism of action. Cutinase is not localized in the mitochondrial fraction but in the cytoplasm of the cell.

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CAFFEIC ACID, A SUBSTANCE WITH AUXIN ACTIVITY FOUND IN EXTRACTS FROM COLEUS RHENALTIANUS

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(*received January 17th, 1961*)

In our study on the abscission of debladed petioles carried out with *Coleus rhenaltianus* (Vendrig, 1960), we detected in extracts from the leaves an auxin that could not be regarded as an indole derivative. It was found that this compound gives a positive response in the *Avena*-curvature test, and that it retards the abscission of debladed petioles of Coleus.

Dettweiler (1942) had extracted from *Coleus* internodes supplied with an IAA (β-indole acetic acid) preparation an acid stable auxin. The production of this auxin was thought to be enhanced by IAA.

In the present investigation we repeated the experiments of Dettweiler, and we tried to identify his auxin by means of the chromatographic method, as it seemed to us that this auxin might be identical with the auxin which we ourselves had extracted from the leaves.

In some preliminary experiments, the same chromatographic separation method was used as in our preceding study (Vendrig, 1960). Besides some spots due to indole derivatives, we especially noticed two spots on the chromatograms which in ultra violet light showed a blue fluorescence, and it appeared that the compounds eluted from these spots gave auxin reaction when used in the *Avena*-coleoptile-section test. The same compounds were also detected in the acid fraction of an extract obtained from untreated plants. The u.v. spectra of both compounds were practically the same as that which was found earlier for the auxin extracted from the leaves. In consequence of this result we decided first to analyse in detail the compounds present in plants that had not been treated with IAA.

Method

The plants were cultivated in a glasshouse under long-day conditions. Cuttings were used from the same clone as in our preceding experiments. In each experiment circa 300 g. leaves + stems (fresh weight) were finely ground in a tissue homogenizer with boiling ethanol. After the homogenate had been left for 18 hours at 4° C, it was filtered, and the filtrate concentrated under reduced pressure. The residue was 6 times extracted with about 30 ml. aq. dest. at 40° C. These aqueous extracts were shaken with peroxide-free ether, and each of the ether fractions was 3 times shaken with 20 ml. of an 8 % $NaHCO_3$ solution. The joint bicarbonate fractions were acidified

with 1 N HCl to pH 4.0. The acidified solution was shaken 4 times with ether. After drying over anhydrous Na sulphate, the ether was evaporated.

The residue was dissolved in 2 ml. methanol. This methanol solution was spotted on the start line of a chromatogram (Whatman no. 4, 50 × 50 cm). After equilibration the chromatogram was developed with an isopropanol/water mixture (5:1 v/v) by means of the descending method.

The spots were detected in long wave u.v light (blue fluorescence). They were eluted with ether as well as with ethanol.

The ether eluate was evaporated, and the residue dissolved in a 1 % glucose solution. This solution and some dilutions were tested on auxin activity in the Avena-coleoptile-section test (according to RIETSEMA, 1951); 5 ml. of solution were used for a series of 10–20 sections.

The alcoholic eluates were further purified over aluminium oxide (Merck), and the perfectly clear filtrate was used to determine the u.v. absorption spectrum by means of a *Zeiss* spectrophotometer. After evaporation under reduced pressure the residue was dissolved again in a 1 % glucose solution and tested on auxin activity.

AUXIN ACTIVITY OF THE UNKNOWN COMPOUNDS

On chromatograms developed with isopropanol/water the Rf-values of the two compounds proved to be 0.79 and 0.84. In Table I the

TABLE I

Growth rate of *Avena*-coleoptile sections in a solution of 1 % glucose and the eluates obtained from a chromatogram (spots at Rf 0.79 and 0.84) of the acid fraction from an extract of *Coleus*. Length of the sections 3 mm. Growth recorded at the end of 24 hours. Number of sections between brackets.

Exp. nr.	Increase in length (eye-piece micrometer units)				
	Dilution of the eluate				
	control	1:1	1:10	1:100	1:1000
1 Rf. 0.79	16.8 ± 1.59 (15)	20.8 ± 1.50 (14)	26.2 ± 1.09 (16)	20.9 ± 1.60 (10)	—
2 Rf. 0.79	18.7 ± 1.41 (14)	39.6 ± 2.72 (16)	22.3 ± 1.96 (13)	—	—
3 Rf. 0.79	16.6 ± 1.34 (13)	—	26.2 ± 1.73 (14)	25.7 ± 1.92 (11)	—
4 Rf. 0.79	8.4 ± 0.89 (10)	23.9 ± 1.86 (9)	16.7 ± 2.31 (8)	—	—
1 Rf. 0.84	13.0 ± 0.72 (15)	6.8 ± 0.50 (15)	19.4 ± 0.76 (17)	—	—
2 Rf. 0.84	8.4 ± 0.89 (10)	21.2 ± 2.60 (9)	20.4 ± 1.04 (10)	11.7 ± 1.13 (9)	9.3 ± 1.42 (9)
3 Rf. 0.84	5.2 ± 0.36 (10)	12.4 ± 0.77 (10)	11.2 ± 0.70 (10)	9.4 ± 0.73 (10)	—
4 Rf. 0.84	5.0 ± 0.45 (10)	8.5 ± 0.43 (10)	10.8 ± 0.58 (10)	8.4 ± 0.62 (10)	—

results are recorded of some experiments on the auxin activity of the eluates obtained from these spots. It appears that both compounds enhanced the elongation growth of the coleoptile sections. The diluted solutions too were significantly active.

Identification of the compounds

In this case too we have tried to identify these physiologically active compounds with indole derivatives. However, all chemical reactions on indole derivatives (Linser and Kiermayer, 1957) yielded a negative result.

The two purified alcoholic eluates show (after dilution) the same u.v. absorption spectrum, with a maximum absorption at $\lambda = 2900$ Å and $\lambda = 3250$ Å. Eluates that had not been purified, exhibited a slight shifting of the maximum absorption.

This spectrum resembles some spectra given by flavonoid compounds (Geissman, 1955), and indeed, if we added a solution of neutral lead acetate to the alcoholic eluates, a yellow precipitate was produced. Spraying the chromatograms with this reagent resulted also in the appearance of two yellow spots.

Another useful qualitative test for flavonoid compounds is that of Shinoda (1928), in which the compound is treated with magnesium and concentrated hydrochloric acid. However, with this reagent no colour developed.

Only few of the flavonoids and related compounds listed by Geissman give a blue fluorescence in ultra-violet light and are at the same time colourless in visible light. 3′4′ Dihydroxy-flavone satisfies these conditions, but this substance has another absorption spectrum.

The following experiments were carried out to prove that the auxin activity exerted by the eluates is actually due to the compounds which give a yellow precipitate with neutral lead acetate.

The yellow precipitate suspended in ethanol was centrifuged, and the ethanol removed. The precipitate was washed with aq. dest. and centrifuged again. To eliminate the lead, diluted sulfuric acid was added. After filtration, the filtrate was neutralised with K-biphosphate to a pH 2.0, and extracted with ether. After drying and evaporating the ether over anhydrous Na-sulphate, the residue was dissolved in a 1 % glucose solution. Table II shows the results of some experiments with *Avena*-coleoptile sections in which some dilutions of the regenerated compound were tested on their auxin activity. With the compound at Rf 0.84 similar results were obtained.

Other known compounds which give a yellow precipitate with neutral lead acetate and which fluoresce blue in u.v. light, are some phenylpropane derivatives, e.g. chlorogenic acid (Paech and Ruckenbrod, 1955). The u.v. absorption spectrum of chlorogenic acid given by Björkman and Holmgren (1960) agrees very closely with the spectra of our unknown compounds. Hoepfner's reagent (1 % $NaNO_2$ in 10 % acetic acid) and $FeCl_3$ give identical colour reactions with chlorogenic acid and with our unknown compounds.

However, commercial chlorogenic acid appeared to have another

TABLE II

Growth rate of *Avena*-coleoptile sections in a solution of 1 % glucose and the regenerated lead precipitate (eluate obtained from the spots in a chromatogram at Rf 0.79). Length of the sections 3 mm. Growth recorded at the end of 24 hours. Number of sections between brackets.

	Increase in length (eye-piece micrometer units)				
	Dilution of the extract				
Exp. nr.	control	1:1	1:10	1:100	1:1000
1	13.4 ± 1.22 (10)	—	—	21.3 ± 1.74 (10)	15.1 ± 1.73 (9)
2	4.3 ± 0.80 (12)	9.1 ± 1.04 (15)	—	7.6 ± 1.22 (12)	8.5 ± 0.57 (13)
3	6.2 ± 0.53 (14)	13.9 ± 0.90 (15)	9.2 ± 0.70 (15)	8.1 ± 0.98 (15)	—

Rf-value (in isopropanol/water: 0.46). Moreover, the elongation growth of Avena coleoptile sections was only slightly enhanced by chlorogenic acid in a 1 % glucose solution. This appears from the experiment recorded in Table III.

TABLE III

Growth rate of *Avena*-coleoptile sections in a solution of 1 % glucose and 0.001–10.0 μg/ml chlorogenic acid. Length of the sections 2 mm. Growth was recorded at the end of 24 hours.

Concentration chlorogenic acid μg/ml	Increase in length (eye-piece micrometer units)	Number of sections
0	9.8 ± 0.72	17
10	12.3 ± 0.74	21
1	12.7 ± 0.70	18
0.1	14.5 ± 0.76	21
0.01	9.2 ± 1.02	18
0.001	8.7 ± 0.89	18

The possibility could not be ruled out that the depside is hydrolysed to quinic acid and caffeic acid. It is known that caffeic acid can act synergistically on IAA and on α-naphthyl acetic acid (REINDERS-GOUWENTAK and SMEETS, 1947; HEMBERG, 1951). According to these authors caffeic acid alone, however, is either slightly inhibitory or inactive.

Caffeic acid too gives a yellow precipitate with neutral lead acetate (due to the two hydroxy groups on the ring). Moreover, we noticed that caffeic acid has the same Rf-value as one of our unknown compounds (0.84 in isopropanol/water). The same correspondence in Rf-value is found when water-saturated ethylacetate is used as a solvent. However, we could detect only one spot in ultra-violet light, if butanol/acetic acid/water (5/2/2 v/v) was used as a solvent. Both caffeic acid and the compounds of our extract had in this case a Rf-value of 0.81.

On chromatograms developed with isopropanol/water only one spot was found, and this proved to have the Rf-value of caffeic acid, when the extract was treated with sulphuric acid.

From leaves of *Solidago virgaurea* Björkman and Holmgren (1960) extracted besides chlorogenic acid a second compound with the u.v. spectrum of caffeic acid and, if the chromatogram was developed with butanol/acetic acid/water, with the same Rf-value. After hydrolysis

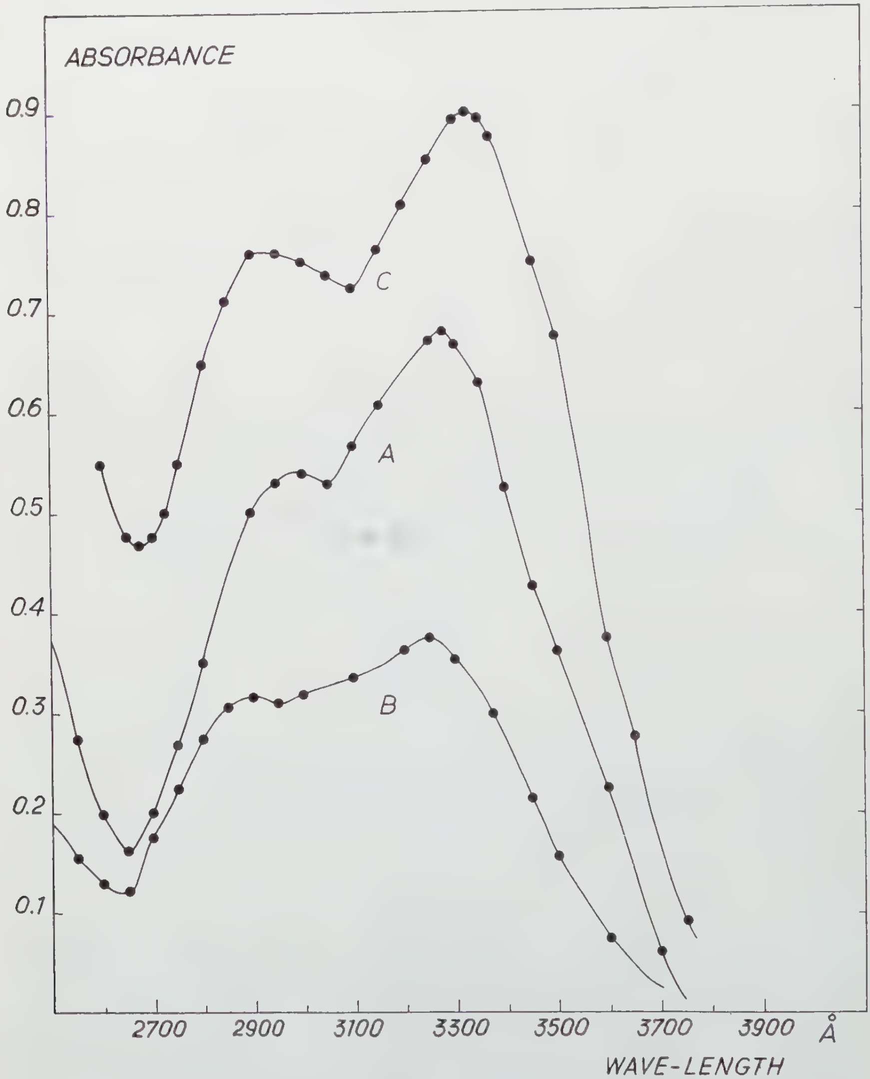

Fig. 1. Ultra-violet absorption spectra of commercial trans-caffeic acid (A) and of the eluates obtained from the spots at Rf 0.79 (B) and 0.84 (C) of a chromatogram developed with isopropanol/water. The substances were dissolved in ethanol.

with hydrochloric acid caffeic acid was obtained as the sole fluorescent substance. According to the authors, their second compound might be identical with iso-chlorogenic acid.

The u.v. absorption spectra of caffeic acid and of our compounds purified from an extract, are reproduced in Fig. 1.

On our chromatograms developed with isopropanol/water, the two fluorescent compounds give the same colour with the following spraying reagents

a) 2 % $FeCl_3$: grey
b) Ammonia vapour: yellow (for a short time)
c) Ammoniacal silver nitrate: black
d) 2.4. Dinitrosalicylic acid in 2 % NaOH: yellow
e) Bariumhydroxyde: yellow.

Auxin activity of caffeic acid

As we now have identified one of the compounds from the acid fraction of a *Coleus* extract with caffeic acid, and the other one as a related substance, it is reasonable to expect that commercial caffeic acid too will show auxin activity in the *Avena* coleoptile section test.

Indeed, caffeic acid, either alone or combined with 0.5 % glucose, greatly enhances the elongation growth of the sections; however, only when applied in low concentrations. Caffeic acid concentrations of 30–100 μg/ml were slightly inhibitory or inactive. Some results are recorded in Table IV. The results of identical experiments with cinnamic acid are recorded in the same table.

Discussion

Bentley (1958) defines auxins as growth regulators which at low concentrations induce cell enlargement. Most of the bioassays that are used to detect auxins in plant extracts are based on this interpretation.

The use of paper and column chromatography has revealed the existence of many unknown substances with auxin activity. Bentley (1958) lists a number of unknown compounds from acid and neutral fractions of ethereal and alcoholic extracts.

The unknown auxin which is present in an ethereal extract from *Coleus* leaves (Vendrig, 1960), is now recognized as consisting of two substances which could be identified by us respectively with caffeic acid and with a related compound.

Caffeic acid has been found in many plants (Tomaszewski, 1960), and is usually considered as a growth inhibitor (Akkerman and Veldstra, 1947). In pedicels of tomatoes caffeic acid is either inactive or exerts but a slight inhibitory effect on cell enlargement. Combined with α-naphthyl acetic acid it has a synergistic effect on the growth hormone (Reinders-Gouwentak and Smeets, 1953). According to these authors caffeic as well as ferulic acid would have some influence on the transport of the natural growth hormone. In tomato the compounds would exert a synergistic effect on applied α-naphthyl

TABLE IV

Growth rate of *Avena*-coleoptile sections in a solution of caffeic resp. cinnamic acid (trans-isomers) in aq. dest. or with 0.5 % glucose. The substances were added 6 hours after the sections had been made and immersed in aq. dest. Length of the sections 2 mm. Growth was recorded at the end of 18 hours. Number of sections between brackets.

			Increase in length (eye-piece micrometer units)			
			Concentration of the acid (μg/ml)			
Exp. nr.	% glucose	Acid	0	3	1	0.5
1	0	caffeic	3.8 ± 0.45 (16)	5.3 ± 0.41 (19)	5.7 ± 0.35 (21)	4.3 ± 0.34 (18)
	0	cinnamic	3.4 ± 0.36 (16)	3.3 ± 0.44 (20)	3.2 ± 0.52 (15)	2.2 ± 0.36 (18)
2	0	caffeic	1.2 ± 0.28 (14)	3.4 ± 0.27 (14)	5.0 ± 0.34 (14)	3.7 ± 0.27 (18)
	0	cinnamic	1.6 ± 0.37 (17)	2.5 ± 0.36 (15)	2.4 ± 0.42 (17)	2.1 ± 0.38 (17)
3	0	caffeic	1.5 ± 0.21 (11)	4.0 ± 0.38 (12)	3.7 ± 0.38 (14)	3.0 ± 0.33 (13)
4	0.5	caffeic	14.2 ± 0.83 (17)	20.0 ± 0.63 (20)	20.1 ± 0.58 (19)	21.8 ± 0.61 (21)
	0.5	cinnamic	14.8 ± 0.57 (24)	13.0 ± 0.84 (14)	12.0 ± 0.92 (12)	13.0 ± 0.80 (12)
5	0.5	caffeic	10.0 ± 0.50 (16)	15.7 ± 0.62 (18)	15.8 ± 0.68 (17)	15.0 ± 0.35 (15)
	0.5	cinnamic	8.3 ± 0.56 (16)	7.8 ± 0.57 (11)	9.5 ± 0.68 (13)	9.8 ± 0.85 (15)

acetic acid, only in those cases where there is no natural growth hormone transport.

HEMBERG (1951) found that caffeic, ferulic and traumatic acid had a synergistic effect on β-indole acetic acid in the *Avena*-curvature test. This author found no effect with caffeic acid alone (at concentrations between 4.4–52 μg/ml), but these concentrations may have been too high to give a positive response. In the *Avena*-coleoptile-section test we found auxin activity only at concentrations between 3 and 0.01 μg/ml.

In peas the content of polyhydroxy cinnamic acids (ferulic, caffeic and chlorogenic acid) proved to be increased after the plants had been sprayed with a solution of gibberellic acid (KÖGL and ELEMA, 1960). According to these authors the polyhydroxy cinnamic acids would act as inhibitors of the IAA-oxydase system, and the "auxin sparing" effect of gibberellic acid would thus be an indirect one.

In our opinion it is not excluded that caffeic acid acts as a true auxin. This can be concluded from our experiments in which the elongation growth of the oat sections proved to be enhanced by caffeic acid while no other auxins were present in the surrounding fluid.

To rule out the possibility that caffeic acid acts synergistically with natural auxin, we will have to carry out experiments with auxin-starved coleoptile sections. In this way NEUMANN (1960) recently could prove that coumarin can act as a stimulator of elongation growth. However, the site of action of this growth regulator in the cells is thought to be different from that of IAA.

VELDSTRA and BOOY (1949) formulated the structural requirements for cell-elongation activity as:

a) a basal ring system (non polar part) with interface activity,
b) a carboxyl group (polar part), or, in general, a group of acidic character, in such spatial position with respect to the ring system that on adsorption of the active molecule to a boundary this functional group will be situated as peripherally as possible.

Introduction of hydrophilic substituents into the lipophilic nucleus would result in inactive or weakly active compounds, as in that way more symmetrically constructed hydrophilic substances are obtained (VELDSTRA, 1953).

Of the stereoisomeric cinnamic acids only *cis*-cinnamic acid is physiologically active (VELDSTRA, 1944).

With these considerations in mind, it is not to be expected that caffeic acid (3′4′ dihydroxy cinnamic acid) would surpass cinnamic acid as a growth substance. The introduction of hydrophilic substituents into the lipophilic nucleus results in a more symmetrically constructed compound, and its auxin activity would thus be diminished. At this moment we had at our disposal only the commercially obtainable cinnamic acid and caffeic acid. They proved to be the more stable *trans* isomers. From these two acids only caffeic acid proved to be able to act as an auxin in our experiments. This means that the introduction of hydrophilic substituents (OH-groups) into the lipophilic nucleus results in a more active compound. Moreover, it points out that the *cis* configuration is not necessary for growth activity.

Experiments with the *cis* isomers are now in progress. In future we hope to obtain more details on the steric configuration of the natural caffeic acid and of related compounds, on their occurrence in different plant parts and on their physiological function in plant metabolism.

The use of chromatographic methods in auxin research has revealed the existence of many substances with auxin activity. Now, the question arises to what extent these substances may be identified with cinnamic acid and related compounds. We have found these compounds also in the neutral fraction of the alcoholic extract. This means that dividing an extract in an acid and a neutral fraction is not fully satisfactory.

SUMMARY

In the alcoholic extract prepared from leaves and stems of *Coleus rhenaltianus*, two substances were detected which in the *Avena*-coleoptile-section test showed auxin activity. These compounds fluoresced blue in ultra-violet light. One of the compounds could be identified with *trans*-caffeic acid. The other one is a related compound, giving the same absorption spectrum and colour reactions.

The physiological activity of *trans*-caffeic acid is discussed in connection with the activity of *trans*-cinnamic acid.

ACKNOWLEDGEMENT

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AN ADDITION TO THE GENUS PHORADENDRON (LORANTHACEAE)

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Even at the beginning of my work on the genus *Dendrophthora* (Loranthaceae) it seemed clear that *D. poeppigii* Van Tieghem occupied an anomalous position in that genus. Sometime ago it occurred to me that, while within *Dendrophthora* I could not point out any close relatives, several species of *Phoradendron* showed great similarities. In general habit, vaginae cataphyllares, and even inflorescence structure *Phoradendron caesalpiniae* Ule, *P. surinamense* Pulle, and *P. linearifolium* Eichler (Trelease, *Phoradendron*, 1916, plates 225b, 226, and 181, respectively) are exceedingly similar to *Dendrophthora poeppigii*. The latter species is also aberrant because of the fact that it grows at low elevations, this in contrast to all other continental species of *Dendrophthora*.

It is no surprise, therefore, to find that the species under consideration is a *Phoradendron* and not a *Dendrophthora*. Krukoff 6011 (see below) has clearly bilocular anthers, and there is no reason to believe that other collections will differ in this respect. Thus once again the seemingly weak distinction between the two genera, namely the number of locules in the anther, appears to be vindicated. A recombination to *Phoradendron*, then, becomes necessary. As Trelease's monograph does not, of course, refer to this species, and Van Tieghem does not provide a sufficient description, a formal treatment follows.

Phoradendron poeppigii (Van Tieghem) Kuijt, n. comb.

Basionym: *Dendrophthora poeppigii* Van Tieghem, Bull. Soc. Bot. France **43**: 182. 1896.

Illustrations: Kuijt, Acta Bot. Neerl. **8**: 510. Fig. 2a (center). 1959. Present paper, Figs. 1 and 2.

An apparently squamate species of extraordinary appearance because of its exceedingly long and slender, terete internodes. The squamate condition perhaps more apparent than real, as leaf scars are sometimes visible below lateral branches; leaves possibly very small, deciduous? Each long internode, whether precurrent or lateral, has its base sheathed by a vagina cataphyllaris; up to 5 similar vaginae are present on inflorescences. Phyllotaxy decussate throughout, including that of the cataphylls; basal appendages of lateral branches

Fig. 1. *Phoradendron poeppigii* (v. Tiegh.) Kuijt, Krukoff 6011, GH.

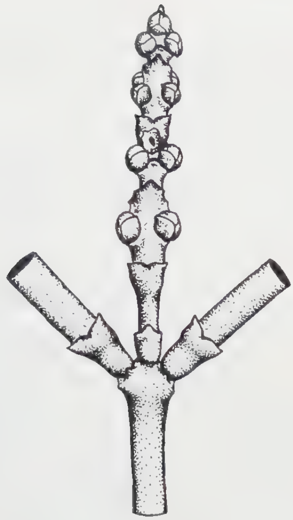

Fig. 2. A terminal inflorescence of *P. poeppigii* (Krukoff 6011, K), with supporting node and portions of lateral branches (× 2).

in median plane, prophylls scarcely discernable. Spikes often lateral, but also terminal, then producing a forked habit. Spikes with up to 5 or 6 fertile internodes, each internode ca. 4 mm long in flower, about 3 flowers per fertile bract, only the apical flower staminate and falling away early; lateral flowers pistillate only. Berries round-ovate, reddish-brown when dry. Inflorescence type 1a or 1b? Monoecious. Type: Poeppig, with illegible data except for "Brasilia Borealis" (P) is in all likelihood the specimen which Van Tieghem saw, and should be regarded as the type.

Specimens seen:

Krukoff 6011, Brasil, State of Amazonas, Manicore, near Bella Vista (F, G, GH, K, MO, NY, S, U, US); Ule 5249, Brasil, Marary, Río Iuruá (G, K); Weir 13, Brasil, State of Amazonas, São José de Amatory, River Amazonas, on *Hevea brasiliensis* (F, GH, MO, NY, US); Weir s.n., Brasil, Puiheiro Island, on *Hevea brasiliensis* (F, GH, MO, NY, US).

The nearest relatives are probably the three other species of *Phoradendron* mentioned above. Another species which is certainly related is *P. platycaulon* Eichler (cf. Kuijt, Acta Bot. Neerl. **8**, Fig. 7a. 1959; Trelease, *Phoradendron*, 1916, plate 227), as is indicated by a very similar spike and an identical sex distribution along the spike. The fact that obviously related species must be culled from widely separate subdivisions in Trelease's treatment does little credit to his system.

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EINIGE BRIEFE VON HUGO DE VRIES
MIT EINEM FACSIMILE IM TEXT

Einbegleitet von P. FRÖSCHEL
(*Botanisch Laboratorium, Utrecht*)

(*eingegangen am 19. Januar 1961*)

Die im Folgenden wiedergegebenen Briefe aus der Feder von Hugo de Vries kamen auf folgende Weise in meine Hände:

Als ein aus der Wiener Schule hervorgegangener Botaniker kam ich mit dem Vererbungsforscher und Professor an der Wiener Hochschule für Bodenkultur Erich (von) Tschermak natürlicher Weise öfters in Kontakt. Bei dem grossen Unterschiede von Alter und Rang jedoch und bei dem respektvollen Abstand, den ich zu wahren hatte, beschränkten sich diese Kontakte auf Diskussionen gelegentlich der Sitzungen der zoologisch-botanischen Gesellschaft und hörten völlig auf als ich nach Beendigung des ersten Weltkrieges für zwei Dezennien aus dem wissenschaftlichen Leben ausschied.

Merkwürdig genug, kam es just durch meine Auswanderung aus Österreich zu einer neuen, nun aber dauernden und viel innigeren Kontaktnahme. Als meine Emigrationsreise durch die gastfreundliche Aufnahme in das von G. L. Funke geleitete pflanzenphysiologische Institut der Universität Gent ihr Ende fand, war ich des Glaubens, das Schicksal hätte zum ersten Male einen österreichischen Botaniker nach Gent verschlagen. Das war indess ein Irrtum. Denn schon im Jahre 1900 weilte der junge österreichische Landwirtschaftsingenieur Erich (von) Tschermak in Gent, um die dort so hochstehende Blumenkultur zu studieren. Es war in Gent, wo er den Plan fasste und ausführte, Kreuzungsversuche zu unternehmen, die, wie man weiss, zur Wiederentdeckung der in Vergessenheit geratenen Mendelschen Vererbungsgesetze führten.

Ich konnte, als Nachfolger Funkes, zweimal mitwirken, um Tschermak die wohlverdienten Auszeichnungen der Universität Gent zu sichern: eine Huldigungsadresse aus Anlass des 50ten Jahrestages der genannten Wiederentdeckung, das Ehrendoktorat anlässlich seines 85ten Geburtstages.

Als ich im Sommer 1960 Tschermak meine Übersiedlung von Gent nach Utrecht zur Kenntnis brachte, schrieb er mir unter dem 5. September 1960: "Mit Holland verknüpfen mich schöne Jugenderinnerungen. Im Jahre 1898 besuchte ich von Gent aus den "Botanischen Pabst" [1]) De Vries, dem ich damals glücklicher Weise nicht verriet, dass ich mich mit Kreuzungsversuchen an Erbsen beschäftigte, sonst hätte er ja gesagt: Denn wandeln auch Sie auf Mendels Spuren und

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[1]) "Pabst" wird in Österreich im Sinne von Autorität verstanden, nicht im Sinne von autoritär. Anm. Fr.

ich wäre nur ein Mitarbeiter aber nicht ein Wiederentdecker der Mendelschen Vererbungsgesetze geworden. Er interessierte sich aber sehr, dass ich ein Enkel des Botanikers Fenzl bin. Mit dem lustigen Botaniker Lotsy war ich sehr befreundet. Er vertrat damals wie Kerner die Ansicht, dass neue Arten nur durch Bestardierung entstanden seien. Sirks, jetzt in Groningen, traf ich auf vielen Vererbungskongressen, ebenso die sympathische Tine Tammes, die so schöne Arbeiten mit Lein gemacht hat. Auch mit Hagedoorn bin ich oft zusammengetroffen. Jetzt bin ich noch mit den Herren Prof. Dorst und De Haan und Wellensiek in Wageningen in Verbindung. In meinen Publikationen erwähnte ich auch die Holländer Giltay, Pitsch, Broekema und De Mol. Vielleicht interessieren Sie diese Daten."

Und auf eine Ansichtskarte, die ich ihm von Velp sandte, antwortete er unter dem 19. September 1960: "In Velp bei Arnheim war ich nach dem Internat. Gartenbaukongress im Jahre 1923 Gast in dem schönen Hause des Generalsekretärs der Amsterdamer Akademie der Wissenschaften Pr. Lotsy mit meiner damals schon recht leidenden ersten Frau. Er war ein sonniger, reizender Mensch, der mich sehr gerne hatte. Seine sowie die Briefe von De Vries habe ich alle aufgehoben."

Daraufhin fragte ich Tschermak an, ob er bereit wäre, einige dieser Briefe an Holland abzutreten, ich würde für deren würdige Unterbringung Sorge tragen.

Tschermak, der im 90. Lebensjahre steht, antwortete unter dem 26. November 1960: "Ich verteile jetzt vor meinem Ableben sehr gerne Briefe hervorragender Botaniker und Zoologen, sowie Pflanzenzüchter und Interessenten, sie würden ja sonst ungenützt verloren gehen." Und er hatte diesem Briefe 8 Schreiben von De Vries beigelegt mit der Bemerkung dass "sich De Vries bald nach dem Erscheinen seines 2-bändigen Werkes "Die Mutationstheorie (1901–1903)" nur mehr ganz wenig mit Bastardierungsfragen beschäftigte und daher unser Briefwechsel bald verstummte."

Ich möchte diese Einbegleitung nicht abschliessen, ohne einer besonderen Stelle dieses Briefes Tschermaks zu gedenken. Sie lautet: ". . . Es ist auch interessant, dass ich erst in den letzten Jahren darauf gekommen bin, dass unter den Vorfahren Mendels der Name Nitschmann aufscheint (vergleiche Seite 174 meiner Memoiren), der auch in dem Ahnenpass der Familie Tschermak vorkommt. Es war nämlich der Grossvater meines Vaters mit einer Johanna Nitschmann aus Littau verheiratet. So habe ich noch mehr Veranlassung des grossen österreichischen Naturforschers Gregor Mendel in Ehrfurcht zu gedenken."

Es besteht also die Möglichkeit einer Verwandtschaft zwischen Gregor Mendel und Tschermak und einer Vererbung des Interesses für Vererbungsfragen.

Die Originale der hier folgenden 4 Briefe und 4 Karten von De Vries wurden dem "Rijksmuseum voor de Geschiedenis der Natuurwetenschappen" einverleibt.

Briefkarte, Amsterdam; 31. Oktober 1898.

Sehr geehrter Herr Doctor,

Hoeflichen Dank für Ihren freundlichen Brief und die Zusendung Ihrer Abhandlung, welche ich mit um so grösseren Interesse gelesen habe, als ich selbst auch ähnliche Besuche bei Gärtnern in Erfurt und sonst gebracht habe.

Sehr angenehm wird es mir sein, wenn Sie mir gelegentlich Nachricht über Ihre Bastardierungsversuche machen wollen. Auch bin ich gerne bereit Ihnen evtl. auf das Beste zu antworten wenn Sie mir Fragen vorlegen wollen.

Hochachtungsvoll
Ergebenst
w.g. H. DE VRIES

Brief, Amsterdam, 18. Juni 1900.

Sehr geehrter Herr Doctor,

Empfangen Sie meinen herzlichen Glückwunsch bei Gelegenheit Ihrer Habilitation und meinen besten Dank für die gefällige Zusendung Ihrer Habilitationsschrift. Wie Sie wohl erwarten dürfen habe ich diese mit grösstem Interesse studiert. Es ist sehr merkwürdig, dass die solange gänzlich vergessene und unrichtig gewürdigte Arbeit Gregor Mendels jetzt gleichzeitig von so verschiedenen Seiten in den Vordergrund der Forschung gestellt wird, und es ist mir eine grosse Freude zu sehen, dass die verschiedenen Meinungen und Forschungen auf dasselbe Ziel hinausgehen.

Zu einer eigentlichen Verwertung Ihrer hochwichtigen Resultate werde ich erst im zweiten Bande meines Buches über die Mutationstheorie kommen. Denn der erste Band handelt nur von der Entstehung der Arten, der zweite aber von den Bastardierungsprozessen. Meine erste Lieferung ist jetzt druckfertig, doch brauche ich bis zum Abschluss der ersten Bandes noch mindestens ein volles Jahr. Inzwischen ist es mir sehr lieb, dass sich das Material fuer den zweiten Band zu häufen anfangt. Über Variabilität ist in den letzten 5–6 Jahren in der Botanischen Literatur ein sehr dankenswerter Bestand zu Tage gefördert; in Bezug auf wissenschaftliche Bastardierungen fängt das aber erst an, und es ist mir besonders sympathisch, Sie unter den ersten Pionnieren dieser neuen und zweifelsohne sehr fruchtbaren Forschungsrichting zu begrüssen.

Ich erwarte vorläufig nicht viele Förderung für die Wissenschaft von Kreuzungen zwischen möglichst verschiedenen Arten und finde es kaum Schade, dass Ihre Versuche in dieser Richtung noch keinen Erfolg haben. Ich habe ähnliches mit verschiedenen anderen Pflanzen versucht, komme aber stets wieder dazu, etwaige unerwartete Resultate als Folge unvollständiger Castration bzw. unvollständiger Isolierung zu verwerfen.

Ihre Versuche über unvollständige Fruchtbildung bei Fremdbestäubung fallen mit denen Strasburgers und Anderer zusammen

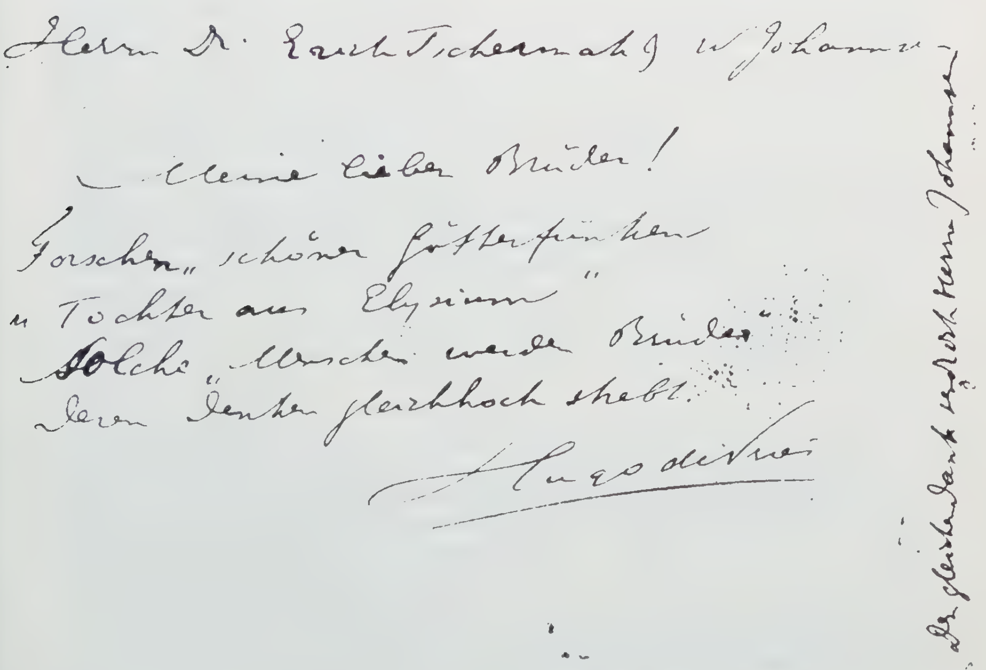
und sollten eigentlich ein ganz von den Bastardierungen getrenntes Gebiet bilden. Es handelt sich dort — scheint es mir — um die Frage, welche Reize die Entwicklung der Frucht ausserhalb des Samens (resp. des Samens ausserhalb des Keimes) beherrschen. Hildebrandt's Versuche über die Folgen der Bestäubung bei Orchis usw. sind hier wohl als das Muster zu behandeln. Aber es ist das doch ganz etwas anderes als die Frage, wie sich die Eigenschaften zweier Arten resp. Unterarten in einem Bastard benehmen.

Kennen Sie Galton's Lehre vom *Midparents*? Ich meine den Satz, dass die Eigenschaften der Kinder bei individueller Variation (also nicht bei Kreuzungen von Arten oder sogenannten Varietäten) von den *mittleren* Eigenschaften beider Eltern bedingt werden, und zwar nach dem Gesetze der Regression. Mir scheint dass dieser sehr fruchtbare Gedanke auf botanischem Gebiete noch viel zu wenig berücksichtigt wird.

Mit freundlichem Grusse hochachtungsvoll,

Ihr ergebener
w.g. H. de Vries

Gehen Sie im Oktober auch nach Paris zum Congrès de Botanique?


Meine lieber Bruder!
Forschen„ schöner Götterfunken
„Tochter aus Elysium"
Solche „Menschen werden Brüder"
deren Denken gleichhoch strebt.
Hugo de Vries

Briefkarte, Amsterdam, 8. August 1901.

Briefkarte, Amsterdam, 8. Oktober 1901.

Sehr geehrter Herr Doctor,

Empfangen Sie meinen besten Dank für Ihren Mendel in handlicher Form. Diese Ausgabe wird gewiss das Interesse an Bastard-Fragen wesentlich verallgemeinern und jedenfalls auch mir die Bearbeitung des zweiten Bandes meiner Mutationstheorie sehr erleichtern.

Sie haben wohl gesehen, dass man in England denselben glücklichen Gedanken gehabt hat und dass die Royal Horticultural Society in ihrem Journal 1901 Vol. 26 p. 192 eine Uebersetzung der beiden Aufsätze Mendels veröffentlicht hat.

Hochachtungsvoll
Ergebenst
w.g. H. DE VRIES

Brief, Amsterdam, 23. November 1901.

Sehr geehrter Herr Doctor,

Empfangen Sie meinen besten Dank für Ihre freundlichen Zusendungen, sowohl für Ihren Brief und die wichtige Abhandlung als für die Photographie Mendels' welche ja ein jetzt sehr wertvolles und seltenes Curiosum ist.

Vieles was Sie mir von Ihrer Lebenslage schreiben, erinnert mich an meine eigenen Anfangs-Jahre. Als ich zu Würzburg studierte konnte ich auch im botanischen Garten nicht den erforderlichen Raum für meine Versuche erlangen, und hatte ich bei einem Gärtner ausserhalb der Stadt ein Stück Land gemietet. Dort habe ich meine ersten Maisversuche über die Xenien u.s.w. angestellt. Der Garten lag aber etwa ½ Stunde vom Institut und von meiner Wohnung entfernt.

Als Sie mich besuchten hatte ich hier allerdings eine ganz bequeme Einrichtung, aber das war erst seit kurzer Zeit, früher waren mein Laboratorium und mein Garten durch eine öffentliche Anlage getrennt, durch welche noch dazu die Strasseneisenbahn lief. Ueber diese hinüber habe ich während fast 10 Jahre alle meine Keimpflanzen in den Garten, und alle Samen und das zu Hause zu untersuchende Material aus dem Garten hinübertragen müssen.

Und Unkraut ausjäten, Pflanzen binden u.s.w. thue ich auch jetzt noch sehr häufig, wenn die Arbeit mehr wird als die Zeit eigentlich gestattet.

In den Weihnachtsferien beabsichtigte ich die Bastard-Literatur vorzunehmen und dann hoffe ich auch Ihre Arbeiten mit mehr Ruhe durchstudieren zu können. Ich hoffe darin vieles zu finden was ich für den Grundsatz der Mutationstheorie, die Lehre von den Einheiten der Eigenschaften, werde benützen können. Eine allgemeine Bastardlehre werde ich selbstverständlich nicht schreiben, da die Bastardfragen für mich nicht selbst Zweck sind, sondern nur das Mittel, um jene Einheiten näher zu beleuchten.

In Hamburg traf ich Herrn Dr. Hjalmar Nielsen, den Vorstand der Versuchstation in Svalöf, Schweden. Sobald ich wieder meine

Zeit frei haben werde um auf eine Woche auf Reisen zu gehen, hoffe ich sehr dorthin gehen zu können. Ich möchte die Anstalt gerne auch mal sehen und bewundern. Uebrigens war es in Hamburg in jeder Hinsicht ausgezeichnet.

Mit höflichen Grüssen

Ihr ergebener
w.g. H. DE VRIES

Brief, Amsterdam, 18. Juni 1902.

Sehr verehrter Herr Doctor,

Besten Dank für Ihren Brief und Ihren neuesten Beitrag. Nur bedaure ich, dass der Text meines Buches bereits abgeschlossen ist — der zweite Band ist in Druck begriffen, und so werde ich Ihre Ergebnisse für diesen Zweck nicht mehr benützen können.

Nach Carlsbad werde ich freilich nicht gehen, ebensowenig wie nach New York im September.

Mein Drahtgitter gefällt mir ganz gut, nur schützt es nicht gegen die Falter von Agrotis segetum, dazu sollte es so engmaschig sein, dass es viel Licht zurückhalten würde. Kleine Käfige mache ich auch in feinem Tuch, gegen Insekten.

Mit besten Grüssen

Hochachtungsvoll
Ergebenst
w.g. H. DE VRIES

Briefkarte, Amsterdam, 24. Februar 1904.

Sehr geehrter Herr Doctor,

Mit bestem Dank bestätige ich den Empfang Ihrer neuen so wichtigen Sendung. Nach England gehe ich nicht, da ich diesen Sommer nach Amerika gehe, wo ich an der Universität von Californien im Sommersemester Vorlesungen halten werde.

Hochachtungsvoll
Ergebenst
w.g. H. DE VRIES

Brief, Amsterdam, 16. Januar 1905.

(Dieser Brief ist gerichtet an Prof. Liebenberg der Wiener Hochschule für Bodenkultur und stellt die Antwort dar auf das Ersuchen, ein Urteil abzugeben über die wissenschaftliche Persönlichkeit Tschermaks, der vom Professoren-Kollegium zum a.o. Professor vorgeschlagen worden war.)

Sehr geehrter Herr College.

In höflicher Beantwortung Ihres sehr verehrenden Schreibens vom 9. Januar glaube ich nicht besser tun zu können, als Sie auf die vielfachen Citate aus Dr. Tschermaks wissenschaftlichen Arbeiten im

zweiten Bande meiner Mutationstheorie und auf das darin mehrfach ausführlich begründete Urteil zu verweisen.

Eine solche öffentliche Äusserung wird ohne Zweifel ein grösseres Gewicht in die Schale legen können als irgendwelche private Meinungsangabe.

Mit vorzüglicher Hochachtung

Ergebenst,
w.g. H. de Vries

Acta Botanica Neerlandica **10** (1961) 209–247

STUDIES ON THE CONVERSIONS OF AMINO ACIDS IN SOIL[1])

W. VAN DRIEL

(*Laboratory for General Botany, Plant Physiology and Pharmacognosy, University of Amsterdam*) [2])

(*received April 7th, 1961*)

CHAPTER 1

INTRODUCTION

Humus is a mixture of compounds formed in the soil by condensation and polymerisation of degradation products from animal and vegetable origin (Schuffelen, 1950). It is a well-known fact that the composition of humus varies widely both in character and relative amounts of its components, depending on the nature of soil, climate, vegetation and moisture conditions. Many years ago nitrogen was already recognized as a constituent of humus (Eggertz, 1889), since all attempts to remove this element as an impurity had been without success. By degradation of the humus, followed by a fractionating procedure and identification of the products some insight was gained about its chemical structure and the structural bond of the nitrogen.

Humus formation occurs principally in the soil outside the living organisms. Undoubtedly the precursors are mainly derived from biological materials, and introductory processes of humification may occur in dying cells. The main processes, however, take place in the soil.

In spite of all attempts to elucidate the chemical processes leading to humus formation, this problem remained essentially unsolved, including the problem of nitrogen incorporation. In the soil a wealth of microbiological and chemical reactions occurs which can transform the degradation products of biological origin in many directions, but only a few of these involve the production of humus. The complex nature and the diversity of these processes are the main difficulties in solving this problem.

Many attempts were made to identify the precursors in the humification process, including nitrogenfree compounds, in particular lignin, carbohydrates and polyquinones, and several nitrogen containing substances, aromatic amino acids being the most important ones in this respect.

These results are partially based on a similarity in chemical structure between compounds isolated from the soil and humus degradation

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[1]) This investigation was carried on from June of 1954 to the end of 1957, under a grant from the "Netherlands organisation for the advancement of pure research (z.w.o.)".

[2]) Present address: Institute for Soil Fertility, Groningen.

products. Partly they rest on investigations of substances which contribute to the formation of dark coloured material in cultures of special soil microorganisms. *Actinomycetes* proved to be most active in this respect. FLAIG c.s. (1952) stated that the aromatic amino acids contribute especially to the synthesis of these products. The other 23 amino acids he tested rarely caused an appreciable dark colouring, only small amounts of brown coloured compounds, at most one tenth of the quantity produced by aromatic amino acids.

The problem of the incorporation of nitrogen in humus compounds has often been considered. It is generally assumed that in nature this process can proceed along two different lines:

According to one view amino acids and related substances would be involved directly in the humification process, without any preceding N-mineralisation. This could happen either under the influence of the action of microorganisms in the soil or in a purely chemo-synthetic way, as has been revealed by laboratory experiments. So RAPER (1928) has been able to show that melanin could be formed out of tyrosine; ENDERS (1943) studied the reaction between glycine and methylglyoxal; WAKSMAN and IYER (1932) synthesized humus-like substances from lignin and protein. All the products formed were dark coloured, but at present it is not quite certain whether these processes contribute considerably to the production of humus, since the conditions in vitro may differ widely from those occurring in nature.

Considering the rapid deamination of amino acids by microorganisms in the soil, others are of the opinion that at first the N-containing organic substances are mineralised under liberation of ammonia. Only in the final stages of the humification process would the ammonia enter the N-free intermediates, formed either by action of microorganisms or synthesized by chemical reactions. In order to establish the importance of this view some investigators have tried to synthesize humus-like substances in this way. MATTSON c.s. (1943) was able to demonstrate the formation of such compounds by the action of ammonia on lignin under laboratory conditions and FLAIG (1950) by the reaction of ammonia with polyquinones. Both reactions were performed under conditions that might occur in soil.

In my opinion, the essential difference between these hypotheses is that, according to the view first mentioned, an important part of the carbon skeleton of the amino acid enters the humus fraction; according to the last mentioned hypothesis amino acid carbon atoms will take part in these syntheses only incidentally. When we started with this investigation little was known about this problem and only a few papers had appeared dealing with the subject. That is why we undertook this work.

CHAPTER 2

SURVEY OF LITERATURE

2.1. The presence of free amino acids in soil

Before discussing the conversion of amino acids in the soil, we will consider their occurrence.

Many attempts have been made to demonstrate the presence of free amino acids in soil (Bremner, 1952; Okuda ,1954; Sowden c.s., 1953; Sowden, 1957). In the early investigations these were found only in acid peat soils. Fowden, Dadd and Pearsall (1953) demonstrated paperchromatographically the presence of a few amino acids in extracts of this type of soil. These extracts were obtained by pressing out the soil sample and by concentrating the extract a hundred fold in vacuo at room temperature above sulfuric acid. The total quantity of amino acids present amounted to maximally 3 μg per gram of air dry soil. In nearly all samples serine, glycine, alanine, glutamic and aspartic acid were found. The presence of threonine, leucine-isoleucine, valine, β-alanine, γ-aminobutyric acid, asparagine, glutamine and some basic amino acids could occasionally be demonstrated. However the analytical methods for determining amino acids were improved considerably in recent years. With these highly sensitive methods it was possible to detect free amino acids in nearly every type of soil. Putnam and Schmidt (1959), Schmidt, Putnam and Paul (1960), Paul (1959), and Simonart (1954) investigated several neutral and alkaline soils. Instead of water they extracted the soil with 70–80 % ethanol, as this resulted in a higher yield. Paul (1959) extracted with 0.1 N $Ba(OH)_2$ and 0.5 N ammonium acetate; in this way an even larger number of amino acids could be detected. After concentrating to a 1000-fold they found 5–25 amino compounds in quantities of 0.05 μg–0.5 μg per gram of soil. This was much less than Fowden c.s. (1953) had found in acid peat soil.

In soils fertilized with glucose and sodium nitrate up to 40 different amino compounds could be detected in concentrations 10–50 fold as high as those in unfertilized soils.

Payne, Rouatt and Katznellson (1956) noticed a brownish colouring when the soil extracts were concentrated in vacuo at 40° C. With the procedure usually followed losses in amino content were reported. No decrease occurred when the extracts were concentrated by freeze-drying. The highest amount of free amino acids found in unfertilized soil has been 0.5 μg per gram of soil.

2.2. The combined amino acids in soil

Amino acids are the principal constituents of the soil nitrogenous material. This fraction gives on acid hydrolysis α-amino-N in a yield of about 25–50 % of the total soil nitrogen. As an example, Rendig (1951) hydrolysed a sample of virgin Miami silt loam with 3 N HCl

for 10 hours at 120° C. Afterwards he was able to isolate the following fractions, containing in terms of the nitrogen originally present: amino acids (including the basic amino acids) 34 %; ammonia released from amides during hydrolysis 20 %; non-basic-non-amino compounds 5 %; inorganic nitrogen compounds 1 %. The remaining fraction, with 40 % of the total N was not hydrolisable and could not be identified. The results of the various analyses depend to a considerable extent on the fractionating procedure used, but when carried out under standard conditions each procedure will give reproducible values.

Up to 1946–1947 the amino acids had to be identified by specific reactions; only a small number could be detected in soil hydrolysates (Kojima, 1947; Waksman, 1938). Much better results were obtained both by paper-chromatography (Bremner, 1946, 1949, 1951, 1955a; Davidson c.s., 1951; Parker c.s. 1952) and by column chromatography (Sowden, 1955; Stevenson, 1954, 1956a, b); with these techniques all amino acids can be separated. Sowden (1956) found 12, Bremner 21 (1950), Stevenson 25 (1956a) and recently Young and Mortensen (1958) 50 ninhydrin-positive substances in soil hydrolysates. These differences probably depend mainly on differences in the various analytical techniques used. However, with both chromatographic procedures a number of compounds, present in soil hydrolysates in low concentrations, remained unidentified.

The question then arises whether the combined amino acids fraction in the soil has a uniform composition. Indeed several authors have stated that the relative amounts of the various amino acids would be constant in many soil hydrolysates (Bremner, 1946, 1949, 1951, 1952, 1954; Sowden, 1956). We must however remark that one meets these views only in older investigations in which less accurate analytical procedures were often applied. At present it is generally recognised that both the total and the relative amounts of the amino acids in the soil hydrolysates may vary. Stevenson (1956b) found important differences depending on the vegetation. In a hydrolysate of soil from an experimental plot exclusively cropped with maize he found 30 % amino-N, expressed as percentages of total soil-N, against 40 % in a permanent grass border round about the same plot. In table 1 a survey is given of his results on the relative amino acid composition of these soil hydrolysates. Carles (1959) also in many soil hydrolysates obtained varying amounts of amino acids.

From all these studies we may conclude that the amino acids in soil hydrolysate originate from divergent compounds. The problem arises: what is the nature of these compounds? It was generally assumed that a considerable percentage of the combined amino acids is derived from proteins.

However these proteins can scarcely be present in an unbound state. It appeared impossible to isolate proteins from soil; chemical identification of these compounds in soil or soil extracts gave negative results, e.g. the amino-end group reaction according to Sanger (Bremner, 1957; Sowden c.s., 1953).

TABLE 1. AMINO ACIDS IN HYDROLYSATES FROM A SILT LOAM SOIL
(Data taken from STEVENSON (1957))

Soil sampled from the top soil. Since 1902 plot A had been cropped with maize, plot B with grass.
Hydrolysis: 16 hours with 6N HCl, at atmospheric pressure. After desalting the amino acids had been separated on a column of DOWEX 50.

Total N: A 0.138 %, B 0.323 % of dry matter
Amino-N-content: A 30.1 %, B 39.9 % of total N

Amino acid	A mg/100 g soil	B mg/100 g soil
glycine	21.8	73.7
alanine	31.7	76.4
β-alanine	5.0	17.3
serine	14.0	73.4
threonine	18.2	81.0
valine	21.3	62.0
leucine	19.5	63.4
isoleucine	16.1	47.7
proline	10.3	51.7
phenylalanine	7.4	23.6
tyrosine	8.0	23.3
aspartic acid	26.0	66.7
glutamic acid	20.0	94.4
ornithine	8.5	8.6
histidine	5.8	6.9
arginine	6.4	17.0
lysine	44.4	70.0
γ-aminobutyric acid	3.5	12.0
α-aminobutyric acid	1.0	3.5
α-ε-diaminopimelic acid	2.5	9.9
methionine	trace	trace
methioninesulfoxyd	11.3	22.7
methioninesulfone	trace	trace
cystine	trace	trace
cysteic acid	5.3	15.9

One could assume that most of the proteins present would be in the living or dead microorganisms; therefore the reactions just mentioned failed to reveal these compounds.

However according to the views of RUSSELL c.s. (1950) and SWABY (1959) the quantity of amino acids, formed by hydrolysis of the proteins of the intact microbes, could at best constitute only a minute fraction of the total amount of amino acids in the soil hydrolysate. They estimated that the countable microorganisms comprise only one percent of the soil organic matter.

More difficulties arise in determining which protein fraction is directly derived from dead microbes, as one cannot follow the estimation procedure of RUSSELL and SWABY in this particular case. One has to assume that this fraction is probably negligible.

In the top soil large quantities of proteins are supplied from plants and animals. These proteins are rapidly converted. There is no evidence of any great difference in the biochemical resistance of the proteins of microbes and those of other plants and animals. The reported occurrence of resistant proteins excreted by some fungi

(Bohonos, c.s., 1942; Morton c.s., 1955) seems to represent an exception. It seems therefore appropiate to assume that microbial proteins will also be rapidly converted.

Practically all the proteins in soil are present in a complex form. This prevented the extraction and isolation of these proteins. Several assumptions have been made about the character of these complexes. Waksman and Iyer (1932) stated that in soil lignin-protein complexes would be present, forming most of the resistant protein fraction. A second assumption is the adsorption of proteins on clay minerals, e.g. on bentonite (Ensminger c.s., 1942, 1944; Lynch c.s., 1956, 1958; McLaren c.s. 1954a, b, 1956, 1958, 1959; Pinck c. s., 1951, 1954). In vitro both lignin- and bentonite-suspensions give rise to a rapid protein adsorption, about three fourths of the maximum adsorption occurring in the first few minutes. The protective action of lignin towards protein is probably due to physical absorption into a dense molecular gel network (Estermann c.s., 1959). The clay-protein complex may derive its resistance to adsorption on the exterior surface or within their expanding lattice layers (Pinck c.s., 1951, 1954). Complexes subjected to in vitro digestion by proteolytic enzymes or by bacteria show a retardation in protein mineralisation, as compared with the conversion of unprotected protein (Estermann c.s., 1959). Complexes with lignin are more resistant than those with bentonite. Added to the soil, both complexes may be decomposed however. The presence of lignin-protein complexes in actual samples of soil could not be demonstrated and up to the present there is no evidence for their existence (Forsyth, 1946; Mattson c.s., 1943; Sowden c.s., 1949, 1955; Stevenson, 1956b).

Allison *et al* (1949) investigated the process of immobilisation of casein-carbon in a mixture of quartz sand, inoculated with a soil infusion, clay minerals having been added in part of the experiment. Adding kaolin to quartz sand brought about a 50 % higher carbon retention in a period of 1.5 to 4 months; after a 12 month period however no differences with quartz sand alone were observed. The clay mineral bentonite, unlike kaolin in having a large base exchange capacity, enhanced the carbon retention in a 1.5 to 4 month period about 100 %; 12 months later the increase was still 50 %. The role of clay protein complexes in the stabilisation of soil protein is therefore significant, although the practical importance is not known.

Complex formation may occur with other suitable materials, e.g. humus. Bremner c.s. (1952, 1955a) isolated several humus fractions, hydrolysed these and found amino acids. After fractionating hydrolysis of a humic acid preparation he observed spots on the chromatogram of peptid-like substances that were not originally present (1955b). Presumably this result may be ascribed to the presence of a protein-humus complex.

It is obvious that part of the fraction of combined amino acids might consist of non-proteinous amino acids, either adsorbed to inorganic colloids, or as a constituent of organic matter. Sowden c.s. (1952) isolating various humus fractions found that 40–70 % of their nitrogen

was α-amino-N. This fraction was purified thoroughly by dissolving and precipitating repeatedly in order to reduce the content of minerals and of intact microbes to insignificant amounts.

The results of the experiments of BREMNER c.s. (1952, 1955a) mentioned on page 214, as well as those of SOWDEN can be partly interpreted by assuming the occurrence of humus-amino acid complexes in soil. It is not likely that the results of these hydrolysis experiments are due exclusively to the presence of humus protein complexes, according to the observations of DRAGUNOW (1958). He found that amino acids of a humic acid preparation were already released after $2^1/_2$ hours of refluxing with 1.5 N HCl. The complete hydrolysis of proteins of *Aspergillus Niger* could be accomplished only after 24 hours refluxing with 6 N HCl. DRAGUNOW ascribes this result to a difference in binding capacity between the amino acids and humus as compared with those in *Aspergillus* proteins. Although nothing was known about the nature of combination of the amino acids in his preparation, it may be assumed that he was engaged in examining humus-amino acid complexes.

SOWDEN (1956) noticed that the relative amounts of the amino acids in soil hydrolysates deviate from those in hydrolysates of proteins of several biological materials. In his soil hydrolysates the relative amount of glycine is higher and that of leucine and methionine less than in hydrolysates of microorganisms, herbage, yeast, vegetables and of grass. However, these results contribute very little to the solution of the problem.

STEVENSON (1956b) found in soil hydrolysates amino acids which were not yet detected in bacterial proteins. Secondary metabolic conversions may occur, yielding amino acids that were not present before. These amino acids would be bound to soil colloids.

In summary, the combined soil-amino acids are spread over the following fractions: a very small percentage is situated in the intact, living or dead microbes. Free resistant proteins are occasionally demonstrated, but only in minute quantities. Nearly all of the amino acids appear either as complex bound proteins, in clay-protein, or humus-protein complexes, or as amino acids bound in the organic soil polymers.

2.3. THE CONVERSIONS OF AMINO ACIDS IN SOIL

In this section we will consider what happens with the amino acids added to soil. Most experiments on the decomposition of these substances have been carried out during investigations of the nitrogen mineralisation, in which attention has mainly been confined to the conversions of the amino acid nitrogen.

The course of decomposition of nitrogeneous compounds in soil depends mainly on environmental conditions. Often the percolation method of LEES and QUASTEL (1946) is used. Although this procedure represents very unnatural conditions, it gives reliable results. A dilute solution of amino acids in water is continuously percolated, well

aerated, through a soil column and the decrease in its amino acid content is determined. Surprisingly the results obtained with the percolation method agrees rather well with those obtained in pot experiments (OWEN, WINSOR and LONG, 1950; SCHMIDT, PUTNAM and PAUL, 1960).

Under aerobic conditions an important part of the amino acid added to the soil is converted into CO_2, H_2O and NH_3 under uptake of an equivalent amount of oxygen (GREENWOOD and LEES, 1956, 1960) The ammonia can be oxidised to nitrite and nitrate, but under conditions of bad aeration and high pH to N_2O, N_2 and NO. These losses by denitrification can amount to one half of the nitrate-nitrogen present. Generally at least half of the amino acids is mineralised. The percentage of mineralised nitrogen depends on the C/N-ratio of the amino acid added, decreasing with an increase in ratio.

Most amino acids disappear completely in 2–9 days as appeared from the paperchromatographic analyses performed by GREENWOOD and LEES (1956), TOMBESI (1953), and SCHMIDT, PUTNAM and PAUL (1960). According to OWEN and WINSOR (1950), QUASTEL and SCHOLEFIELD (1950) and GREENWOOD and LEES (1956) the sulphur containing amino acids cysteïne and methionine were converted very slowly, whereas SCHMIDT, PUTNAM and PAUL (1960) recently reported a rapid decrease of methionine. They suppose that this may be due to different reaction conditions.

Adding a mixture of several amino acids, TOMBESI (1953) and SCHMIDT, PUTNAM and PAUL (1960), sometimes after a few days incubation, detected a new amino acid, probably β-alanine, which had not been present before. GREENWOOD and LEES (1960) however never observed the occurrence of a new amino acid.

The products formed from the amino acids which were not totally mineralised, have not yet been identified. Generally it is taken that these are used for the synthesis of microbial matter. GREENWOOD and LEES (1956) point out that the C/N-ratio of these substances varies between 1.2 and 3.9. They assume that these N-rich products may be considered to be bacterial proteins, but mostly bacterial matter has a C/N-ratio of 5–6.

Under anaerobic conditions the amino acid breakdown is different; leucine, methionine, phenylalanine, proline, hydroxyproline, threonine, tryptophan, tyrosine and valine were found to be fairly resistant. They were decomposed more slowly than alanine, arginine, aspartic acid, cystine, glutamic acid, glycine, histidine, lysine and serine. A casein hydrolysate however was deaminated rapidly and completely. The anaerobic carbon mineralisation yield was very low; most of the carbon was recovered as volatile fatty acids (GREENWOOD and LEES, 1960).

2.4. DYNAMIC ASPECTS OF THE COMBINED AMINO ACID FRACTION IN SOIL

Two important problems concerning the stable amino acid complexes in soil are left: how were these amino acids immobilised, and what is

the rate of their release and breakdown? The second problem is particularly important.

SIMONART and MAYAUDON (1958) showed that not all amino acids in soil organic matter are derived from added proteins. A special humus fraction, incubated with C^{14}-labeled glucose for two months, gave on hydrolysis a considerable amount of labeled amino acids. Obviously the glucose carbon was used for synthesis of bacterial matter; the proteins and amino acids formed were afterwards partly incorporated in the humus fraction. WINSOR and POLLARD (1956) could demonstrate that the C/N-ratios of the immobilised residues, from various products rich in nitrogen, very often reach a value of about 6. This points too to a retention of proteins formed in microbes during the decomposition of the parent material.

It is not an easy matter to determine the rate of decomposition of the stable amino acids in soil. However, by their respiration processes the microbes will rapidly decompose the more unstable fractions of soil organic matter. A transient equilibrium is reached; the rate of production of carbon dioxide and the production of mineral nitrogen compounds are proportional to the rate of breakdown of the 'stable' organic matter. This process largely depends on environmental factors. It can be rapidly enhanced by air-drying and remoistening the soil, leading to a considerable increase in the content of phosphate, ammonia and free amino acids.

The data given do not allow us to determine what part of the supplied organic substances is converted into humus or into stable organic matter. KORTLEVEN c.s. (1960) investigated the relation between the formation and breakdown of humus, the humus content of the soil and the yearly supply of organic material. Depending on environmental and pedological conditions each soil type has an optimal humus content. He was able to demonstrate that, under favourable conditions, 25 % of the total supply of organic material was immobilised yearly. At present only a few factors have been investigated further.

In this paper an attempt has been made to increase the knowledge about these problems.

CHAPTER 3

METHODS

3.1. TREATMENT OF THE INVESTIGATED SOIL

A light sandy soil from the top layer of arable land in the vicinity of VENRAY was used in this investigation. This soil belongs to the diluvial deposits, typical for the southern provinces of the NETHERLANDS, and occurs especially on both sides of the valley of the river MEUSE. The results of an analysis of the soil were: 7 % silt and clay, 90 % sand, 3 % humus, no $CaCO_3$, 0,15 % total N, pH 5,6.

A stock of a hundred kilograms of this soil was stored in a glazed

stoneware open container, buried in the garden, separated from the soil underneath by a layer of washed gravel, and covered with fine-meshed nylon gauze to prevent contamination. The soil was subjected to the naturally occurring rainfall. All seedlings were removed. During storage a nitrification process gradually set in. The soil acidified and growth of moss was observed; at this stage the stock was discarded.

Originally the groundwater level was several meters below the surface of the soil, but under storage conditions this difference was only one meter. In the beginning this change in water level had a large influence on the microbial activity. Therefore the stock had to be left for some time under the new conditions before starting an experiment.

Samples of about one kilogram were taken by means of a soil core of 20 mm ϕ. Each sample was homogenised by rubbing it through a 1 mm sieve, and by mixing it thoroughly. Afterwards the soil was kept in darkness for a few days at a constant humidity and temperature of 23° C. During this period the microbial activity adjusted to the new conditions. In order to reduce changes in the bacterial population due to the treatment, the samples were kept under the same moisture conditions as the stock.

From these samples smaller ones of twenty gram each were taken for the measurements. For this purpose the soil was spread out in a reaction vessel (volume $\pm$ 100 ml.) in a layer of about 1 cm thickness, and 1 ml of water (23° C.) was sprinkled in tiny droplets onto the surface. If substrates had to be added they were dissolved previously in this water. In case of insufficient solubility, the compounds were mixed as a fine powder with the soil, after which 1 ml of water was added as just described. All experiments were performed at a constant temperature (23° $\pm$ 1 ° C.).

From preliminary experiments it appeared that no differences in O_2-uptake, CO_2-production or amino acid conversion could be demonstrated, whether the substrate was added as a solid or dissolved in water. Stirring of the soil sample in order to obtain a better distribution of the substrate gave no improvement in the results; in wet soil mixtures, water logging occurred with mixing and deviating figures resulted. In the method of addition described, mixing is not necessary, and is not recommended on account of the water logging.

3.2. Analytical procedures

The rate of O_2-uptake and of CO_2-production, the amounts of amino acids, keto acids, nitrate, and nitrite, and of ammonium present, the total nitrogen and the water content were determined.

Water content. The water content was calculated from the loss in weight of a sample of 10–20 g after drying in an oven at 105° C for 17 hours. Measurements were carried out in triplicate and the average loss is given in % dry weight. The standard deviation amounted to 1,3 % of the water content.

TOTAL NITROGEN. 5 g of soil was digested according to KJELDAHL with 4 g of selenium mixture (WIENINGER mixture, MERCK) and of 20 ml of concentrated sulfuric acid. The ammonia distilled off in a Parnas Wagner apparatus, was determined colorimetrically with NESSLER's reagent. If nitrate or nitrite was present in the soil, a reduction according to JODLBAUER had to precede the destruction. The standard deviation amounted to 3,5 % of the nitrogen content.

GAS EXCHANGE. The experiments involving O_2-consumption and CO_2-production were carried out in a manometric apparatus according to GORTER (1940). The apparatus consisted of 24 gas burettes, 25 ml each, graduated in 0.05 ml. The vessels had a volume of 100 ml. The temperature of the water bath like that of the room was 23° C ± 1° C. The vessels were mechanically shaken with 30 strokes per minute.

Each vessel was provided with an interchangeable inner vessel. 2 ml of 4 N NaOH was placed in it for CO_2-fixation. At the end of the experiment the inner vessel was connected to a second vessel, containing 10 ml of 25 % sulfuric acid in the main compartment, and allowed to equilibrate for 20 minutes in the water bath before mixing its content with the acid. The CO_2-evolution was measured.

All measurements were performed in triplicate, mean values are given in mMoles per sample of 20 g. The standard deviation of each triplicate, calculated from 30 measurements, was 2,4 % of the O_2-consumption and 1.4 % of the CO_2-production. Larger soil samples would have resulted in smaller dispersion, but technically this could hardly be achieved.

This method enabled us to determine the gas exchange of small soil samples under nearly natural conditions. The soil percolation method of LEES and QUASTEL (1946) also requires samples of 20–50 g but the experimental conditions differ extremely from those in nature. The bacterial processes, normally occurring in very thin water films on the soil particles (BARTHOLOMEV c.s., 1946) now proceed in a large volume of water. A more promising method for the determination of gas exchange makes use of the apparatus of SWABY and PASSEY (1953) modified by BIRCH and FRIEND (1956). Here the oxygen consumed is replenished automatically by means of electrolysis of acidified water; the hydrogen liberated is caught in a gas buret and serves as a measure for the oxygen uptake. Larger soil samples are needed. Although not carried out by the authors it would also be possible to estimate the amount of CO_2 produced at the end of the experiment.

The pH and inorganic nitrogen were determined in the extract obtained by shaking the sample with 5 volumes of 1 M NaCl and afterwards centrifuging at 1700 g for 15 minutes.

PH. The pH was determined in the extract by means of an Electrofact-pH-meter with a glass electrode.

AMMONIA. An aliquot of the extract containing 10–200 μg N was distilled at 100° C with 1 ml of a saturated solution of borax in a

Parnas Wagner apparatus. 30 ml of the distillate was collected in 1 ml 1 N sulfuric acid, made up to 50 ml; then the ammonia was determined colorimetrically at 450 mμ in 25 ml of the solution 1 ml of NESSLER's reagent having been added.

NITRITE and NITRATE. To the remaining solution, the ammonia having been distilled off, 50 mg DEVARDA-alloy and 1 ml 4 N NaOH were added and the nitrite and nitrate present reduced to ammonium. This was determined as described previously.

The measurements were performed in triplicate. The standard deviation of each triplicate determination, derived from 30 soil samples, was 3.3 % for the ammonia values and 4.2 % for the combined nitrite and nitrate values.

Known amounts of nitrate an ammonia added to the soil could be recovered quantitatively. No interference from labile N-compounds giving rise to the production of ammonia by the analytical procedures was demonstrated. Adding 200 times excess of amino acid-N did not disturb the results.

In soils with a high exchange capacity some of the ammonium ions remained bound to the adsorbtion complex after extraction with NaCl. Sufficient recovery could be brought about by extracting repeatedly or by percolation.

COMBINED AMINO ACIDS. To liberate the amino acids combined in the soil the sample was boiled under reflux with 25 volumes of 6 N HCl for 20–24 hours. The suspension was centrifuged and the sediment washed four times with 25 ml of water. The combined solutions were evaporated to dryness in vacuo at 40° C. Water was added and the extract again evaporated. This treatment was repeated until the excess of HCl had almost completely disappeared. The residue was taken up in 25 ml of water, 4 N NaOH was added to produce pH 5, and the mixture filtered and washed with water. The filtrate was diluted to 50 ml.

The desalting was brought about by adsorbtion of the amino acids on a cation exchanger in the H^+-form. (IMAC C 12, ACTIVIT, Amsterdam). The column (100 × 12 mm) was washed with distilled water till Cl^- free. The amino acids were eluted with 1 N ammonia (REDFIELD, 1953). The ammonia was distilled off in vacuo with a slight excess of 0.1 N $Ba(OH)_2$-solution; the barium ions were precipitated with an equivalent amount of sulfuric acid, and the amino acids determined as will be described further.

Hydrolysis of soil can give rise to considerable losses of several amino acids. STEVENSON (1956a) found losses of the aromatic amino acids, of methionine, cysteine and of arginine; LAATSCH c.s. (1953) reported similar losses. A solution containing known amounts of various amino acids was added to the soil and subjected to the same treatment. On the paper chromatogram compared with the control series sometimes a loss of basic amino acids could be demonstrated.

AMINO ACIDS. As NaCl interferes with the measurements water was used to extract the soil for determining the amino acids and keto acids. One extraction with 5 volumes of water sufficed. Unlike PUTNAM and SCHMIDT (1959), but in agreement with LEES and QUASTEL (1946), we did not get any appreciable adsorbtion of the amino acids added to the soil.

In nature and under storage conditions free amino acids were absent; even on a paper chromatogram no traces of amino acids could be detected.

QUALITATIVE DETERMINATION OF AMINO ACIDS. The amino acids were separated by two dimensional descending paper chromatography according to REDFIELD (1953), SCHLEICHER and SCHÜLL 2043 A paper (23 × 23 cm) was used. No special arrangements for temperature constancy were provided. The following solvents recommended by REDFIELD (1953) gave the best results: first direction: methanol, water, pyridine 80–20–4 (V/V), second direction: tertiairy butanol, n-butanone, water, diethylamine 40–40–20–4 (V/V). Only using fresh solvents the relative position of the spots was constant. Shifting and reversing of several spots was noticed using an aged solvent 2.

About 0.04μ mol N of amino acids was spotted on the chromatography paper. After development the paper was dried and steamed for 30–45 minutes to remove the diethylamine quantitatively, which otherwise interferes with the colour reaction. The amino acids were visualized by spraying with a 0.5 % solution of ninhydrin in 96 % ethanol. The paper was heated for two minutes at 105° C.; heating for a longer period interferes with the results by a strong background colouring. The spots appeared to be blue or yellow.

The following amino acids could be separated: arginine, ornithine, lysine, aspartic acid, glutamic acid, asparagine, glutamine, citrulline, glycine, histidine, α-alanine, hydroxyproline, β-alanine, γ-aminobutyric acid, proline, serine, tyrosine, methionine, valine, phenylalanine, threonine, leucine-isoleucine-norleucine and tryptophan. The spots of glucosamine and of α-aminobutyric acid coincided; those of leucine, isoleucine and norleucine partly coincided, likewise those of hydroxyproline and of β-alanine, but the latter varied in colour.

QUANTITATIVE DETERMINATION OF AMINO ACIDS. A non specific colour reagent for the quantitative determination is given by MOORE and STEIN (1948). The reagent consists of 10 mg of ninhydrin (B.D.H., recrystalized from water) dissolved in 0.5 ml methylethylene glycol, mixed with 0.8 mg of stannous chloride (MERCK) dissolved in 0.5 ml 0.4 M citrate buffer solution, pH 5.0. One ml of this reagent was added to a 0.2 ml amino acid solution, which contained 0.5–50 μg amino-N. It was heated for exactly 20 minutes in a boiling water bath, cooled, and diluted with 5 ml 50 % ethanol. The extinction is measured within an hour either at 570 mμ or at 440 mμ (for blue resp. yellow coloured solutions). Ammonia reacting with ninhydrin also, had to be removed beforehand. If only very small amounts were present, this could be omitted by correcting the results.

KETO ACIDS, QUALITATIVE DETERMINATION. Keto acids being unstable, they had to be converted into the stable phenyl hydrazones. Simple mixtures may be separated by paper chromatography, for the analysis of more complex mixtures of the hydrazones they must be reduced to the corresponding keto acids.

The determination of keto acids as hydrazones was carried out using the method of CAVALINI and FRONTALI (1954), in a simplified procedure as no proteins were present. To 30 ml of the water extract (page 221) 1 ml 0.2 % of 2-4-dinitrophenylhydrazine in 2 N HCl was added, and allowed to react for 20 minutes. When the hydrazones were formed, the solution was extracted with ethyl ether until all colouring substances were removed. The combined ether extracts were evaporated under vacuo to dryness, the residue taken up in 1 or 2 ml 1 N ammonia and extracted with an equal volume of chloroform to remove excess reagent and any neutral hydrazones present. An aliquot of this solution, containing 10–50 μg of the keto acid hydrazones was chromatographed descending with the solvent n-butanol, ethanol, water, 40–10–50 (v/v). The phenylhydrazones of the keto acids give yellow spots, brown-purple in ultra violet light. The reagent does not fluoresce. Spraying with alkali resulted in a better differentiation of the spots (TOWERS c.s., 1954).

CAVALINI and FRONTALI (1954) were able to determine keto acids quantitatively by eluting the spots and colorimetric determination.

As keto acids often form two isomers, preventing the analysis of complex mixtures by this method, TOWERS c.s. (1954) and ALFTHAN c.s. (1955) reduced the 2-4-dinitrophenylhydrazones into the corresponding amino acids. These can be identified easier quantitatively.

We prepared and isolated the hydrazones according to TOWERS, but for the reduction and purification the method of ALFTHAN was used. 20 g of soil, 120 ml ethanol, 50 mg 2–4-dinitrophenylhydrazine and 0.25 ml concentrated sulfuric acid were mixed in a blendor and allowed to react for one hour. The mixture was centrifuged and decanted, and the precipitate washed several times with ethyl acetate. The ethanol extract was evaporated to dryness at room temperature. The residue combined with the ethyl acetate extract, filtered, washed with a few ml of water, and extracted with a 1 % solution of sodium carbonate. The alkaline extract was acidified and extracted with ethyl acetate. The solution of the hydrazones was evaporated to dryness at room temperature, the residue taken up in absolute ethanol, and made to 50 ml.

25 ml of this solution was cooled at 0–5° C, 1.5 g tin powder added, after which dry hydrochloric acid gas was led through, under continously cooling, until all tin was dissolved. The reaction mixture was diluted with water, the Sn-ions were removed with H_2S and filtered off. The filtrate contained the amino acids formed out of the keto acids. After evaporating to dryness the procedures for desalting and two dimensional paper chromatography (page 220, 221) were carried out.

In order to establish the absence of amino acids in the original soil

extract several control measurements had to be done, omitting the reduction, or the addition of the reagent, or both.

QUANTITATIVE DETERMINATION OF KETO ACIDS. No method is available to isolate and to determine quantitatively all keto acids present in a complex mixture. Even the method in which the keto acids are reduced to the corresponding amino acids did not give reliable results. According to TOWERS c.s. (1954), considerable losses, up to 40 %, may arise.

In this investigation the total amount of keto acids present was determined as described by WOLF (1955) for plant material. Deproteinization could be omitted. To 3 ml of water extract (page 221) was added 1 ml of a 0.1 % solution of 2-4-dinitrophenylhydrazine in 2 N HCl, at a temperature of 25°C. After 25 minutes, the hydrazones were extracted with 8 ml ethyl acetate during 2 minutes mixing with an air current. The water layer was sucked off using a glass capillairy. The ethyl acetate solution was extracted with 6 ml 10 % Na_2CO_3-solution during two minutes mixing with an air current. It is essential to observe accurately the periods given. 5 ml of the water layer was mixed in a colorimeter tube with 5 ml 1 N NaOH, and colorimetred after exactly 5 minutes at a wave length of 430 mμ.

With this method 90–95 % of the amounts of keto acids added could be recovered. As it appeared that in a given set of experiments the deviation was practically constant, a control series with known amounts of a keto acid added was always measured. With these precautions correct results were obtained.

THE ACCURACY OF THE DETERMINATIONS. The accuracy with which the compounds in the soil could be estimated was given for each analytical method. All operations were performed threefold. The variability between the measurements depends largely on the macrohomogeneity of the soil sample. Technically we were able to obtain samples differing not more than a few percent. The only exception was the determination of the total nitrogen present, which may be attributed to the presence of inhomogeneities rich in nitrogen, such as plant material passing the sieves.

CHAPTER 4

CONVERSIONS OF AMINO ACIDS IN SOIL

It has been demonstrated in a number of studies that the soil is subjected to continuous alteration. Thus while investigating the conversions of substrates added to soil it is essential to take into account the reactions normally taking place.

4.1. THE SOIL INVESTIGATED UNDER THE EXPERIMENTAL CONDITIONS

Changes in the soil structure and in the ground water level, as affected by the pretreatment, alter the rate of the biochemical processes

such as nitrification, denitrification and ammonification. It appeared in the course of this investigation that under storage conditions the pH of the soil dropped and nitrate was accumulated. However the pH changes observed were small. It may be that they are the result of the higher moisture content. In situ this value was much lower than under storage conditions. BARTHOLOMEV c.s. (1951) indicates that nitrification increases with increasing moisture content, provided aeration is not the limiting factor.

It appeared that the variations depend on the season in which the soil samples were taken.

Changes in the nitrate content were also observed once more when the soil was kept under aerobic conditions at constant temperature and humidity. The soil respiration decreased gradually on storage. Previous to this decrease a temporary increase in the CO_2-production must have occurred, caused by the disturbance of the soil structure. In general the ratio $-\Delta O_2/\Delta CO_2$ varies between 0.84 and 1.35.

TABLE 2. GAS EXCHANGE IN COVERSAND SOIL WITH DIFFERENT MOISTURE CONTENTS

Soil sample from VENRAY. O_2-uptake and CO_2-production expressed in μMoles/day/20 g of soil (= 14.6 g of airdried soil). Incubation period 15 days. Temperature 23° C.

Moisture content, %	O_2-uptake, μMoles/day	CO_2-production, μMoles/day
27	5.5	5.4
72	5.6	5.3

Considerable changes in the moisture content of the soil under investigation scarcely influenced the rate of the gas exchange in a 15 day experiment. O_2-uptake and CO_2-production did not alter when the moisture content of the well aerated soil increased from 27 % to 72 %, and a layer of water was situated on the surface (Table 2).

This result simplified the experimental procedure, although this has to be verified with the other conversions studied.

4.2. MINERALISATION OF ALANINE IN SOIL

Amino acids added to the soil are mineralised in 2–9 days. A deamination takes place by which the ammonia splits off; the carbon moiety is disintegrated.

In Fig. 1 the course of the mineralisation of alanine in the soil is given. In all experiments the measurements were performed simultaneously on soil both with and without added substrates. The differences between these measurements were considered to be the result of conversion of substrate. In Fig. 1 and in all following experiments only these differences are given.

Within 3 days the alanine added had disappeared totally, the quantity of mineralised nitrogen remaining constant thereafter. The O_2-uptake increases throughout the whole experiment. This must be

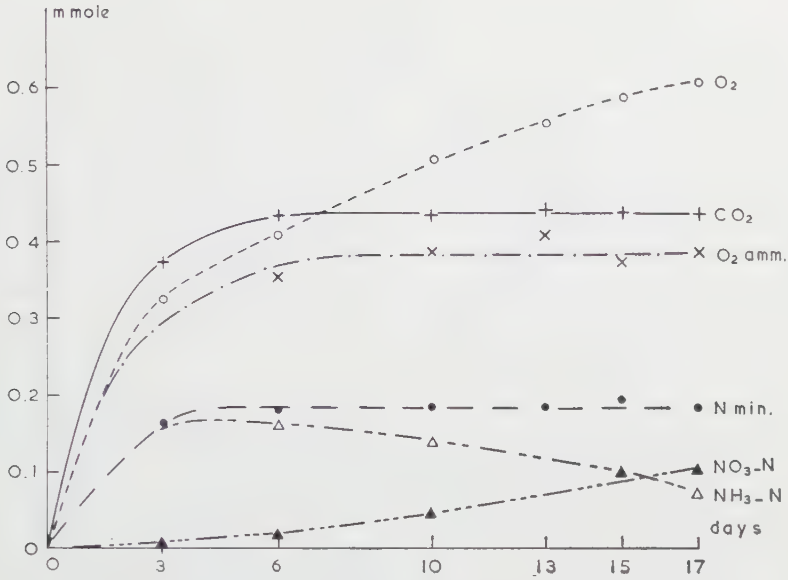

Fig. 1. Mineralisation of alanine in soil. 0.2 mMoles of alanine added to 20 g of coversand soil – Moisture content: 17 % – In this summation curve, the differences are given for soil without addition of alanine—The oxygen uptake is given with and without subtraction of the oxygen needed for the conversion of ammonia into nitrate. Each plot represents the mean of three determinations.

due to the conversion of ammonia into nitrate since the values of O_2-amm. (the O_2 consumed by the ammonification process) appeared to be constant. This value was obtained by diminishing the measured O_2-uptake by the O_2 equivalent of the conversion of NH_3 into NO_3^- calculated from the increase in NO_3-N.

From this experiment it appears that the mineralisation of alanine quickly comes to an end. At that time about 73 % of the carbon and 89 % of the nitrogen was mineralised, total oxidation being postulated. Part of the amino acid-C and N was immobilised, either by accumulation in microorganisms or by formation of "humic" substances.

The ratio between the oxygen used for the conversion of alanine and the carbon dioxide formed amounted to 0.91. By total oxidation of the alanine this value would have been 1.00. Thus the alanine-carbon not immobilised is oxidised for the greater part. In studying the formation of humus, our attention was directed mainly to the C and N that was not mineralised, since only these compounds might act as humus precursors.

The question arises whether the capacity of the soil to convert amino acids in the way described is equal for samples taken at any given time from the stock outside. In Table 3 some observations concerning this are summarised. In general the mineralisation yields are comparable wiht those reported in Fig. 1.

TABLE 3. MINERALISATION OF ALANINE IN SOIL

Soiltype: coversand from VENRAY.— 18–8-1956 brought in experimental garden. Treatment and data: § 3.1. At the beginning of the experiment to each soil sample of 20 g was added either 1 ml of water or 1 ml of a solution of 0.2 mMole alanine. Temp.: 23° C. At the end of the incubation all free amino acids were consumed. The CO_2-production, O_2-uptake and moisture content of the sample, and in the NaCl-extract nitrate-N and ammonium-N were determined. Each value is the average of three parallel experiments.

	storage time of soil before beginning of experiment	month in which experiment started	period of incubation	moisture content of soil	Analysis of 20 g of soil after incubation in the absence of alanine					CO_2-production, O_2-uptake and increase in NH_3-N and NO_3-N as a result of the conversion of 0.2 mMole of alanine[4]).							
										Nitrogen mineralisation			CO_2 production		O_2-amm. uptake		
	days		days	%	pH, end exp.	NO_3-N μMoles	NH_3-N μMoles	CO_2 μMoles/day	O_2 μMoles/day	NH_3-N mMoles	NO_3-N mMoles	%[1])	mMoles	%[2])	mMoles	%[3])	O_2-amm./CO_2 mMole/mMole
A	0	11	20	14	5.0	18	0	8.9	12	0.07	0.10	85	0.42	71	0.43	71	1.01
B	21	1	12	23	5.1	—	—	—	—	0.09	0.08	85	0.43	72	0.40	67	1.08
C	41	2	19	22	5.1	36	0	7.8	7.0	0.08	0.10	89	0.42	70	0.38	63	0.91
D	64	3	8	23	5.1	32	0	8.2	6.9	0.15	0.03	89	0.44	73	0.42	70	0.95
E	27	4	10	21	4.9	36	0	10.7	10.5	0.12	0.05	85	0.45	75	0.43	71	0.96

1) Nitrogen mineralised in % of added alanine-N.
2) Carbon mineralised in % of added alanine-C.
3) O_2-amm. in % of the oxygen necessary for conversion of alanine into CO_2, H_2O and NH_3.
4) Corrected for the control series in absence of alanine.

4.3. Influence of moisture content of soil on the mineralisation of alanine

It has been shown already that within a period of two weeks the O_2-uptake of the soil was not affected by a large change in the moisture content. In Table 4 an experiment is recorded on the relation between the moisture content and the mineralisation of alanine. Up to a moisture content of 30 %, the soil was well aerated; the soil structure did not change, the N-mineralisation was scarcely altered. With a moisture content of about 30 % stirring of the soil has to be avoided,

Table 4. Relation between moisture content of the soil and mineralisation of alanine

Experiment as in Table 3. 0.2 mMoles of alanine added to 20 g. soil. The moisture content was obtained by adding distilled water at 23° C. Incubation 15 days.

Moisture content, %	CO_2-production, O_2-uptake, and increase in NH_3-N, NO_3-N as a result of the conversion of 0.2 mMoles of alanine.					
	nitrogen mineralisation		CO_2-production		O_2-amm.	
	mMoles NH_3	mMoles NO_3	mMoles	%	mMoles	%
22	0.095	0.075	0.43	72	0.40	66
27	0.076	0.086	0.45	75	0.45	75
42	0.104	0.029	0.45	75	0.42	70
72	0.111	0.036	0.44	74	0.41	70

as waterlogging may easily occur then. Above 30 % the structure could not be maintained and the aeration sharply decreased. The N-mineralisation yield increased somewhat, which agreed with the observations of Greenwood and Lees (1960) about the conversions of amino acids in soil under anaerobic conditions. Moreover, a sharp decline in the amount of nitrate formed was observed, the NH_3-content rose, doubtless by the decrease of the NH_3-oxidation rate, and by the decreasing immobilisation. The phenomenon of optimal nitrification by a moisture content approximating the field capacity of the soil has been observed by several investigators. As was expected, the total O_2-consumption decreased when the aeration was disturbed; the value O_2-amm. appeared to be constant.

These results facilitated the technical procedure, since slight changes in the moisture content of the soil gave rise to only small deviations of the total mineralisation. We assumed that a similar relation holds too for other amino acids.

When the soil is subjected to a change in moisture content lasting a relatively long period, the microbial population will change markedly, both quantitatively and qualitatively.

4.4. Influence of the storage period of soil on amino acid mineralisation

As previously mentioned, at the beginning of an experiment the activity of the soil gradually changed under the influence of new

conditions of constant temperature (23° C) and constant moisture content. Therefore the soil was kept for some time previously under conditions similar to those of the experiment. During winter, this acclimatisation had to be extended to several months. Thus we were able to reduce considerably the variability in results.

Beforehand we had to establish that this pretreatment of the soil did not seriously influence the mineralisation process of amino acids. The mineralisation of glutamic acid and of tyrosine were studied, both in concentrations of about equal amounts of carbon. Therefore the C-mineralisation, (but not the N-mineralisation) may be compared. At the end of the experiment the amino acids had totally disappeared and a state of dynamic equilibrium was obtained.

TABLE 5. INFLUENCE OF THE STORAGE OF SOIL ON THE MINERALISATION OF GLUTAMIC ACID AND TYROSINE

Experiment as described in Table 3. Storage of the soil during various periods at constant temperature (23° C) and moisture content (14 %). After these pretreatments amino acids were added. Incubation period: 18 days. Soil samples: 20 g.

Storage period, days	pH at end of experiment	CO_2-production, O_2-uptake and increase in NH_3-N, NO_3-N as a result of the amino acid conversion.						
		Nitrogen mineralisation			CO_2-production		O_2-amm. uptake	
		NH_3-N mMoles	NO_3-N mMoles	%[1]	mMoles	%[2]	mMoles	%[3]
0.12 mMoles of GLUTAMIC ACID added								
0	4.8	0.0018	0.086	73	0.41	69	0.35	64
25	4.8	0.0086	0.067	63	0.39	65	0.40	74
46	5.0	0.0352	0.058	78	0.41	68	0.33	60
0.06 mMoles of TYROSINE added								
0	5.1	0.0058	0.037	65	0.39	66	0.45	71
25	5.0	0.0	0.041	62	0.38	63	0.44	71
46	5.1	0.0049	0.039	67	0.38	63	0.44	71

1) N mineralised in % of added amino acid-N.
2) C mineralised in % of added amino acid-C.
3) O_2-amm. in % of the oxygen necessary for conversion of the amino acid in to CO_2, H_2O and NH_3.

The results are given in Table 5. It was found that for both amino acids the O_2-consumption and CO_2-production were within narrow variation limits independent of the period of the pretreatment of the soil up to 46 days. For the N-mineralisation also no large variations were observed, for tyrosine the values were very nearly constant.

About 71 % of the amino-N and 67 % of the C-atoms of the glutamic acid added were mineralised, the amount of O_2 taken up sufficed to oxidise 67 % of this compound. For tyrosine these results were 65, 64 and 71 % respectively. It is apparent that the degradation products of tyrosine remaining in the soil are partly oxidised.

The only difference brought about by the pretreatment of the soil that could be demonstrated was the decreasing rate of the nitrification processes with glutamic acid. After a pretreatment of three weeks practically all of the 0.12 mMole NH_3 produced was oxidised in 18 days, however after a six weeks pretreatment this was only 60 %.

It has been established in a variety of cases that the activity of the nitrifying microorganisms in soil samples decreases. Adding of ammonia to the sample stimulated the relevant microbial population which counteracts the fluctuations in nitrifying activity. The nitrate formed by this procedure was removed by percolating with water, after which the amino acid conversions could be carried out. In the last few years a similar stimulation of the organisms converting amino acids in soil has also been applied, but we did not follow this procedure as it would have created too unnatural a condition for this investigation and, in any event, the conversion of ammonia into nitrate was rather unimportant for the problem investigated.

4.5. The influence of concentration of amino acids on their mineralisation

Under natural conditions the amino acids are gradually released by hydrolysis of protein. For this reason we had to examine whether the mineralisation yield depends on amino acid concentration. Alanine, glutamic acid and tyrosine were added in various concentrations to soil samples. The results have been given in Table 6. During the incubation period the amino acids disappeared completely.

It will be noted that N-mineralisation increases the higher the amino acid concentration. This holds also for the percentage of N-mineralised. Degradation products of small amounts of amino acids are relatively more fixed than of larger quantities. Comparing equal molar concentrations it appears that the fixation is largest for tyrosine, least for alanine. As was expected the rate of the NO_3-production does not depend on the nature of the amino acid, but on the amount of NH_3 produced (Fig. 2).

The relative C-mineralisation, unlike the N-mineralisation, proceeds independently of the concentration of amino acids.

If an amino acid is immobilised in the soil without changing its C/N-ratio the amount of N in this fraction will be proportional to its amount of C. Graphically this would result in a point on the straight line OA (Fig. 3), where the abscissa represents the ratio of the quantity of carbon-immobilised (in m.atoms) and the number of C-atoms per N-atom in the amino acid molecule, and the ordinate represents the amount of N-immobilised (in m.atoms). The straight line OA is independent of the nature of the amino acid added.

A point deviating from OA would indicate the formation of a product differing in its C/N-ratio from that of the amino acid added.

In Fig. 3 no significant deviations were observed with small amounts of alanine and glutamic acid. When larger amounts of these amino acids were administered, compounds enriched in carbon were produced.

TABLE 6. INFLUENCE OF AMINO ACID CONCENTRATION ON MINERALISATION. Experiment as described in Table 3. Soil sample: 20 g. Moisture content: 21 %. Incubation period 9 days. In exp. A the soil sample was stored for 27 days; in exp. B: 47 days, and in exp. C: 51 days.

Amino acid added, mMoles	pH at end of exp.	NH_3-N+NO_3-N mMoles	%[1]	CO_2-production mMoles	%[2]	O_2-amm. uptake mMoles	%[3]	O_2-amm./CO_2 mMole/mMole
A ALANINE								
0.05	4.9	0.032	64	0.10	70	0.094	63	0.94
0.1	4.9	0.077	77	0.20	68	0.20	67	1.00
0.2	5.2	0.170	85	0.45	75	0.43	72	0.96
B GLUTAMIC ACID								
0.05	4.9	0.033	66	0.17	69	0.13	58	0.75
0.1	5.0	0.073	73	0.27	72	0.28	62	0.77
0.2	5.2	0.164	82	0.36	75	0.67	74	0.89
C TYROSINE								
0.025	4.8	0.010	40	1.16	70	0.088	37	0.47
0.05	4.8	0.024	48	0.29	64	0.28	59	0.96
0.1	4.8	0.064	64	0.60	67	0.66	69	1.10

Column group heading: CO_2-production, O_2-uptake and increase in NH_3-N+NO_3-N as a result of the amino acid conversion.

1) N mineralised in % of added amino acid-N.
2) C mineralised in % of added amino acid-C.
3) O_2-amm. in % of the oxygen necessary for conversion of the amino acid into CO_2, H_2O and NH_3.

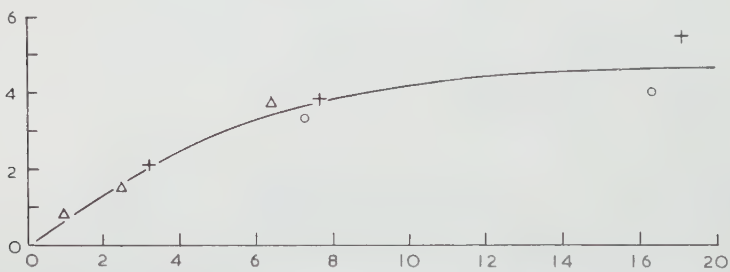

Fig. 2. Relationship between nitrogen mineralisation and nitrification in twenty days. Data in Table 6— Abscissa: NH_3-N + NO_3-N in milliatoms after an incubation period of twenty days— Ordinate: NO_3-N in milliatoms after the same period — + = alanine; o = glutamic acid; Δ = tyrosine.

The experiments with tyrosine resulted in a graph situated above the line OA, indicating a relatively high N content of the end-products formed.

The amino acids, particularly tyrosine, were not immobilised unaltered, as appeared from amino acid estimations of soil hydrolysates.

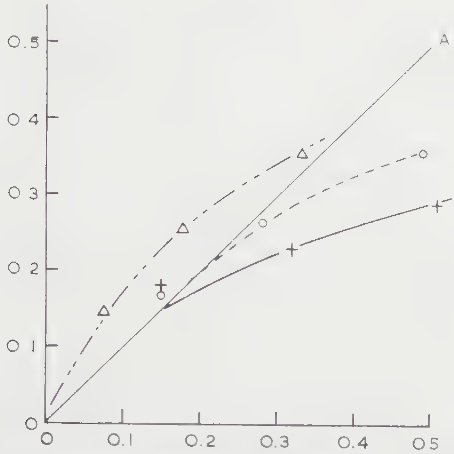

Fig. 3. Relationship between nitrogen and carbon immobilisation. Data in table 6—

Abscissa: $\frac{\text{milliatoms C-immobilised}}{\text{number of C-atoms added per N-atom}}$

—Ordinate: milliatoms N-immobilised— + = alanine; o = glutamic acid; Δ = tyrosine.

Shortly after the tyrosine had been added to the soil it could not be detected in the hydrolysates.

4.6. Comparative investigation on the mineralisation yields of amino acids

As was shown in Fig. 1, mineralisation of amino acids stopped in a few days, the percentages of carbon and nitrogen mineralised remained constant. These values were not affected by changing the storage and incubation periods of the soil, nor by altering its moisture content. Modifying the amounts amino acids added did not affect the percentage of the carbon mineralised.

However the mineralisation of nitrogen depended on the amounts of amino acid added. In order to eliminate this disturbing interference we had to add in each experiment equal amounts of amino acid-N. In this way we were able to compare the maximum mineralisation of various amino acids in each experiment. The results obtained might be disturbed if differences arose in the permeability of the cellwalls or in the biological activity of the relevant microorganisms for the various amino acids investigated. This will happen only in experiments too short to establish a dynamic equilibrium. For this reason we continued the experiments until this state of equilibrium had been reached.

The apparatus enabled us to determine simultaneously in triplicate the mineralisation of six amino acids; in the experiment to be described here twelve amino acids were investigated in two groups of six each. All environmental conditions remaining constant, except that the storage period differed two weeks, but this did not affect the results.

Fig. 4a

Fig. 4b

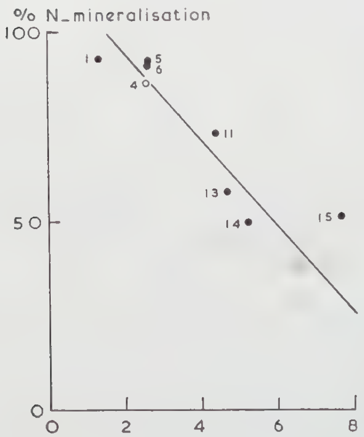

Fig. 4c

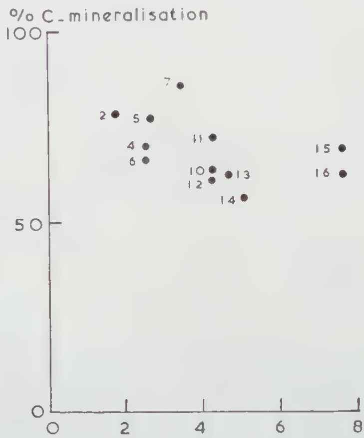

Fig. 5

Fig. 4. Relation between relative nitrogen mineralisation and the C/N-ratio of the added amino acid. Experiment A: data in Table 7 — Experiments A, B and C: Abscissa: C/N-ratio of the amino acid in g/g-

$$\text{Ordinate: } \frac{NH_3\text{–}N + NO_3\text{–}N.}{\text{animo acid-N.}} \cdot 100\ \%$$

1: arginine; 2: glycine: 3: histidine; 4: α–alanine; 5: serine; 6: lysine; 7: aspartic acid; 8: γ– aminobutyric acid; 9: threonine; 10: valine; 11: glutamic acid; 12: proline; 13: tryptophan; 14: isoleucine; 15: tyrosine; 16: phenylalanine.

Fig. 5. Relationship between relative carbon mineralisation and the C/N-ratio of the added amino acid. Data in Table 7 — Abscissa: C/N-ratio in g/g of the amino acid — Ordinate: CO_2-C·100 %/amino acid-C.

TABLE 7. MINERALISATION OF SEVERAL AMINO ACIDS

Experiments as described in Table 3. Amino acid-N in all experiments: 0.1 milli-atoms. Moisture content: 18 %. Soil samples: 20 g. Incubation period: 10 days.

Amino acid added, mMoles		C/N-ratio, g/g	Storage period of soil before incubation, days	pH at end of exp.	CO_2-production, O_2-uptake and increase in NH_3-N+NO_3-N as a result of the amino acid conversion.								C/N immobilised residue, g/g
					NH_3-N+NO_3-N		CO_2-production		O_2-amm.-uptake		O_2-amm./CO_2		
											total oxidation	found	
					mMoles	%[1]	mMoles	%[2]	mMoles	%[3]	mMoles	mMoles	
glycine	0.1	1.71	12	5.5	0.098	98	0.15	74	0.09	61	0.75	0.53	23
alanine	0.1	2.57	12	5.1	0.097	97	0.21	70	0.20	67	1.00	0.90	24
serine	0.1	2.57	24	5.2	0.092	92	0.23	77	0.18	73	0.83	0.79	7
lysine	0.05	2.57	24	4.9	0.091	91	0.20	67	0.21	60	1.17	1.04	10
aspartic	0.1	3.42	12	5.1	0.097	97	0.35	86	0.20	66	0.75	0.58	16
valine	0.1	4.29	12	5.2	0.072	72	0.32	63	0.38	63	1.20	1.19	6
glutamic	0.1	4.29	12	5.0	0.081	81	0.36	72	0.28	63	0.90	0.79	6
proline	0.1	4.29	24	5.1	0.065	65	0.31	62	0.31	56	1.10	0.99	5
tryptoph.	0.05	4.71	24	5.0	0.060	60	0.35	64	0.33	58	1.05	0.95	4
ileuc.	0.1	5.14	12	5.3	0.058	58	0.34	57	0.40	54	1.25	1.17	5
tyrosine	0.1	7.71	24	5.1	0.056	56	0.62	69	0.63	66	1.06	1.01	5
phen.ala.	0.1	7.71	24	5.1	0.045	45	0.56	62	0.64	64	1.11	1.14	5

1) N mineralised in % of added amino acid-N.
2) C mineralised in % of added amino acid-C.
3) O_2-amm. in % of the oxygen necessary for conversion of the amino acid into CO_2, H_2O and NH_3.

The C/N-ratios of the amino acids tested ranged from 1.7 to 7.7.

In Fig. 4 (exp. A) and Table 7 the results of this experiment are summarised. Fig. 4 (exp. B and C) shows the results from further experiments. In Fig. 4 and 5 the ultimate values of the mineralisation of the nitrogen and of the carbon are plotted against the C/N ratios of the amino acids added. It appears that the relative nitrogen mineralisation decreases with increasing C/N-ratio. OWEN, WINSOR and LONG (1950) have already described this phenomenon.

The impression was gained that the results obtained with practically all amino acids could be fitted by a straight line. In all experiments performed tyrosine was an exception, being situated above this line.

When this straight line is extrapolated to the C/N-ratio zero it intersects the ordinate at a mineralisation value above 100 % in our experiments, whereas in the experiments of OWEN, WINSOR and LONG (1950) and of GREENWOOD and LEES (1956) this value is less than 100 %. We were not surprised. In our experiments, total nitrogen mineralisation has already been obtained with a C/N-ratio of 1.5–2.4. In this poor sandy soil only a minute quantity of carbon compounds is available for enzymatic disintegration. For a significant nitrogen fixation, carbon compounds have to be added. The authors cited used fertile garden soil for their experiments. Under these conditions relatively large amounts of carbon are available, which immobilise the ammonia formed.

In practically all the experiments the respiratory quotients obtained agreed fairly well with the expected values if the amino acids were completely oxidised (Table 7).

The C/N-ratio of the residue is the result of a complex system of reactions. The amino acid may be used to synthesize bacterial material

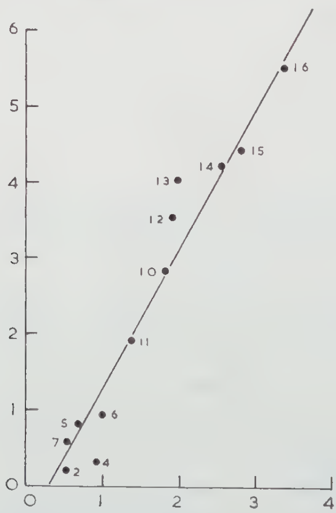

Fig. 6. Relationship between nitrogen- and carbon immobilisation. Data in Table 7 — Abscissa: mMoles C-immobilised; — Ordinate: mMolesN-immobilised.

or to produce resistant humus compounds. The values of the C/N-ratios calculated for the non-mineralised residues are, apart from those for glycine, alanine, lysine and aspartic acid remarkably constant (Fig. 6). The high values found for the amino acids just mentioned have less significance; because of the observed extremely low nitrogen immobilisation, the experimental error has a disproportionate influence.

CHAPTER 5

CONVERSIONS OF KETO ACIDS IN SOIL

5.1. Introductory remarks

The preceding chapter dealt with the decomposition of amino acids in soil. However the pathways of their decomposition were not discussed.

Research on microbiological conversions of amino acids has revealed several pathways of breakdown. For the most part, amino acids are converted by deamination or transamination into the relevant keto acids and ammonia, but other pathways have also been reported (Waksman, 1952). Lichstein (1951) has given a summary of the various pathways, of the intermediates that appear and of the enzyme systems taking an active part in the oxidative deamination.

We investigated whether keto acids are to be considered as intermediates during these microbiological reactions in the soil. This could be done by comparing the conversion rate of the keto acid and that of the relevant amino acid, or by identification of the keto acid as an intermediate in the breakdown of the amino acid.

5.2. The conversion rate of a keto acid and the relevant amino acid

The decomposition rates of alanine and of sodium-pyruvate, added in equimolar quantities to the soil, were determined by the methods described at pages 221 and 223.

Table 8 shows that pyruvate is broken down more rapidly than alanine. With α-ketoglutaric acid, phenylpyruvic acid and p-hydroxyphenylpyruvic acid, corresponding results were obtained.

We also checked whether the conversion of sodiumpyruvate occurs chemically or microbiologically. For this purpose the test was repeated with sterilised soil samples, obtained by heating three times with intervals of 24 hours at 120° C. for 30 minutes, adding a sterile pyruvate solution, obtained by sterilising for 30 minutes at 120° C. The results have also been given in Table 8. The pyruvate was decomposed by the soil only very slowly, so that it is of no significance under natural conditions.

If keto acids were intermediates in amino acid breakdown one would expect that the carbon of a keto acid would be mineralised for a percentage corresponding to that of the relevant amino acid. This

TABLE 8. COMPARISON OF THE RATES OF BREAKDOWN OF ALANINE AND PYRUVIC ACID

Soil sample described in Table 3. Moisture content 17 %. Temperature 23° C. Experiment A: 20 g of soil + 1 ml of water + 0.1 mMoles of alanine. Experiment B: 20 g of soil + 1 ml of water + 0.1 mMoles of sodiumpyruvate. Experiment C: 20 g of sterile soil (heated three times, at intervals of 24 hours, at 120° C for 30 minutes) + 1 ml of water (sterile) + 0.01 mMoles of sodium pyruvate (sterile). Control experiment: 20 g of soil + 1 ml of water. Each experiment was performed in triplicate.

Period of incubation, days	A alanine, mMoles	B Na-pyruvate, mMoles	C Na-pyruvate, mMoles
0	0.1	0.1	0.1
1	0.070	0	0.097
2	0.035	0	—
3	0	0	—
4	0	0	0.055
5	0	0	0.045
11	0	0	0.045

TABLE 9. COMPARISON OF THE BREAKDOWN OF AMINO ACIDS AND THE CORRESPONDING KETO ACIDS

Experiments as described in Table 3. Soil samples: 20 g. Moisture content: 22 %. Incubation period: 9 days.

	Substrate	mMoles	pH at end of exp.	NH_3-N + NO_3-N mMoles	%1)	CO_2-production mMoles	%2)	O_2-amm.-uptake mMoles	%3)
A	alanine	0.1	5.2	0.076	76	0.26	66	0.22	72
	Na-pyruvate+$NH_4$4)	0.1	5.3	0.082	82	0.23	59	0.15	60
B	alanine	0.1	5.2	0.068	68	0.25	65	0.19	63
	Na-pyruvate+$NH_4$4)	0.1	5.2	0.066	66	0.21	54	0.13	50
C	glutamic acid	0.1	4.6	0.071	71	0.32	64	0.26	58
	α-ketoglut.acid	0.1	4.4	—	—	0.30	60	0.18	45
	α-ketogl.+$NH_4$4)	0.1	4.4	0.106	106	0.28	56	0.24	60
D	glutamic acid	0.1	4.6	0.071	71	0.32	64	0.26	58
	α-ketogl.+$NH_4$4)	0.1	4.4	0.101	101	0.28	56	0.23	58
E	glutamic acid	0.1	5.1	0.070	70	0.34	68	0.30	67
	α-ketogl.+$NH_4$4)	0.1	4.9	0.081	81	0.31	62	0.21	53
F	glutamic acid	0.1	4.9	0.069	69	0.36	72	0.26	58
	α-ketogl.+$NH_4$4)	0.1	4.8	0.076	76	0.31	62	0.22	55
G	tyrosine	0.067	5.4	0.049	73	0.33	55	0.36	57
	pOHphen.pyruv.	0.067	5.5	—	—	0.06	10	0.06	10
	pOHph.pyr.+$NH_4$4)	0.067	5.1	0.071	106	0.07	11	0.10	17
H	tyrosine	0.1	4.9	0.044	44	0.36	71	0.60	67
	pOHph.pyr.+$NH_4$4)	0.1	4.9	0.099	99	0.18	21	0.15	17

1) N mineralised in % of added substrate-N.
2) C mineralised in % of added substrate-C.
3) O_2-amm. in % of the oxygen necessary for conversion of the substrate into CO_2, H_2O and NH_3 (if present).
4) keto acid + equivalent amount of NH_4Cl.

has been tested for alanine and pyruvate, glutamic acid and α-ketoglutaric acid and for tyrosine p-hydroxyphenylpyruvic acid (Table 9).

The result obtained was unexpected. Always with a keto acid the increase in CO_2-production was less than the increase with the corresponding amino acid. The differences were small for alanine—pyruvate and for glutamic acid-α-ketoglutarate, but very large for tyrosine-p-hydroxyphenylpyruvic acid.

This result can not be ascribed to a specific toxic action of the keto acids on the soil micro flora in the concentrations used. Otherwise one could not understand their rapid breakdown.

We could assume however that, by the rapid conversion of the keto acids, an intermediate is accumulated having a toxic action on the microorganisms in the soil. The breakdown of the corresponding amino acid would lead to the formation of the same compounds. But as this breakdown proceeds more slowly this substance would be present in a lower concentration. The toxic action would be less and

TABLE 10. RELATIONSHIP BETWEEN KETO ACID CONCENTRATION AND THE MINERALISATION PROCESS

Experiments as described in Table 3. Soil samples: 20 g. Moisture content: 21 %. Incubation period: 9 days. Data further to Table 6. These experiments were performed simultaneously with those in Table 6.

Substrate,[4] mMoles	pH at end of experiment	CO_2-production, O_2-uptake and increase in NH_3-N + NO_3-N as a result of the conversion of keto acid.					
		NH_3-N+NO_3-N		CO_2-production		O_2-amm.uptake	
		mMoles	%[1]	mMoles	%[2]	mMoles	%[3]
PYRUVIC ACID							
0.05	4.9	0.044	88	0.095	64	0.049	38
0.1	5.0	0.088	88	0.177	59	0.078	31
0.2	5.3	0.176	88	0.375	63	0.24	47
α-KETO GLUTARIC ACID							
0.05	4.8	0.035	70	0.14	57	0.069	34
0.1	4.8	0.075	75	0.30	60	0.18	45
0.2	4.7	0.174	87	0.58	58	0.42	53
P-HYDROXYPHENYLPYRUVIC ACID							
0.025	4.8	0.0193	77	0.08	35	0.056	25
0.05	4.7	0.048	96	0.107	24	0.078	17
0.1	4.6	0.103	103	0.14	16	0.15	16

1) N 'mineralised' in % of added NH_4Cl.
2) C mineralised in % of added keto acid-C.
3) O_2-amm. in % of the oxygen necessary for conversion of the keto acid into CO_2 and H_2O.
4) keto acid + equivalent amount of NH_4Cl.

one had to assume that the microorganisms would be able eventually to convert this compound into a non-toxic end-product.

This assumption was tested by investigating the effect of various concentrations of keto acids (Table 10). It appeared that within the range of the concentrations investigated neither pyruvic acid, nor α-ketoglutaric acid differ in their relative carbon mineralisation.

p-Hydroxypyruvic acid gave a different picture. Increasing concentrations of this keto acid inhibits the relative carbon mineralisation. This effect may be due to a toxic action of intermediate formed by the breakdown of this keto acid. The toxicity decreases by diminishing the amount of substrate added.

As the carbon immobilisation depended on the quantity of available nitrogen we have added an equivalent amount of ammonium-ions, in the form of NH_4Cl (Tables 9 and 10). It appears from Table 9 that this altered the carbon mineralisation very little. In several experiments some of the ammonium ions added were immobilised.

5.3. Keto acids in soil

From the preceding paragraph it may be concluded with fair certainty that keto acids act as intermediates during the amino acid breakdown in soil. The deviations found in the metabolism may be due to concentration effects, as has been shown for p-hydroxyphenylpyruvic acid. We then attempted to detect keto acids during the metabolic process.

5.3.1. The occurrence of free keto acids in soil. We had to investigate whether free keto acids are present in the untreated soil samples. By paper-chromatographic analyses of their extracts, treated with 2-4-dinitrophenylhydrazine (page 222) a spot with a high rF-value was observed, indicating the presence of a free keto acid.

As the reaction was scarcely significant we repeated the analysis with a soil extract concentrated tenfold. The spot was observed again and showed very clearly the brown-purple fluorescence characteristic for hydrazones of keto acids. The spot area is not influenced by the concentration of the reagent, which excludes the possibility that the keto acid was artificially formed by decomposition of the reagent by the soil.

However the assumption that the hydrazone of the unknown substance was derived from an acid carbonyl compound had to be proved. This was done by reducing the hydrazone. To this end a large amount of the hydrazone compound was prepared from the soil sample by the procedure described by Towers c.s. (1954). The extract obtained was concentrated tenfold and reduced according to Alfthan c.s. (1955) (page 222). The product obtained was afterwards isolated by paper-chromatography.

On the chromatogram a spot was visible, lacking on the control-chromatogram from an untreated soil sample. The spot gave all the characteristic reactions of an amino acid. The relative place and shape

of the spot were characteristic and reproducible (Fig. 7) but did not correspond to the amino acids mentioned on page 221. We did not isolate the compound in a pure state, so further analysis had to be omitted.

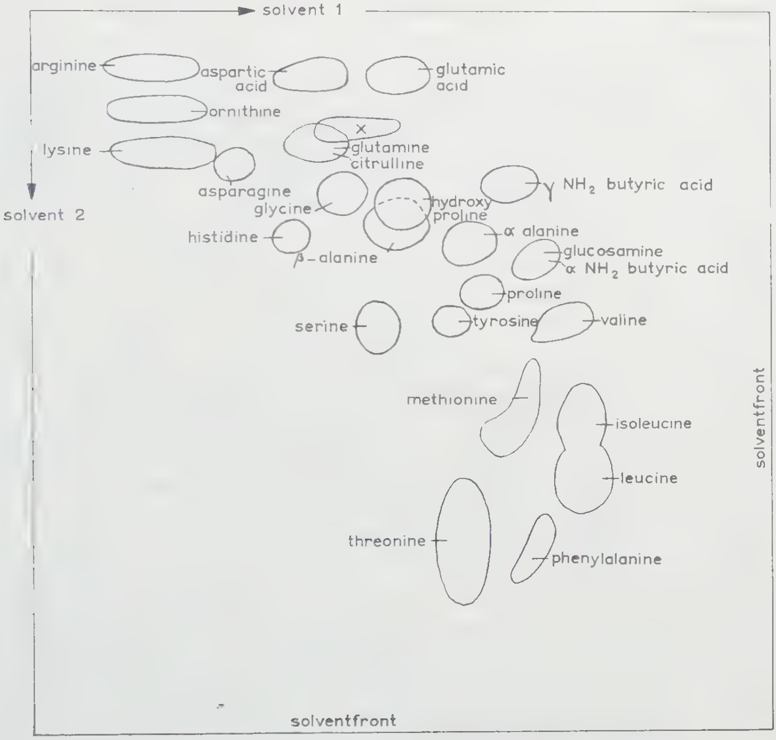

Fig. 7. Paperchromatogram of 23 known amino acids and the amino acid "X", prepared from the keto acid present in the untreated soil. Solvent I: methanol — water — pyridine 80–20–4 (V/V) Solvent II: butanone-2 — tertiairy butanol — water — diethylamine 40–40–20–4 (V/V).

5.3.2. The occurrence of keto acids during the decomposition of amino acids in soil. During the experiments in which the decomposition of amino acids by soil was investigated, the presence of keto acids in the soil extract was regularly determined by paper-chromatography (page 222) and by colorimetry (page 223). The following amino acids had been tested: glycine, alanine, serine, valine, isoleucine, aspartic acid, glutamic acid, lysine, proline, phenylalanine, tyrosine and tryptophan. The soil extracts were sampled during the period in which the amino acid had not been completely converted, since this is the period in which one may expect that keto acids will be present.

In none of the soil extracts a keto acid could be demonstrated colorimetrically. Thus less than 0.5 % of the amino acid could be present in the form of a keto acid.

By the paperchromatographical test incidently several spots were observed. However since several spots of keto acids appeared as the result of the decomposition of only one amino acid one has to assume that these keto acids are secondary metabolic products, and not direct derivatives.

5.4. Conclusion

The occurrence of keto acids as metabolic intermediates during amino acid breakdown could not be demonstrated directly. However, by comparing the conversion of amino acids and keto acids it however seems highly probable that keto acids have to be considered as intermediates in the breakdown of amino acids.

CHAPTER 6

DISCUSSION

The purpose of this investigation was to study conversions of amino acids in soil including the immobilisation of these acids and their degradation products. We intended to perform the experiments under conditions differing as little as possible from the natural ones. Experiments of this kind may be considered as a starting point of every investigation on humus formation.

Several precautions had to be taken to satisfy these requirements. In order to experiment under conditions similar to those in the field, the moisture content of the soil had to remain unchanged. Many investigators have not taken this precaution. Generally they air-dry the soil before starting an experiment, because this facilitates the treatment of the samples. However, this drying causes a selective dying of specific groups of microorganisms. Moreover the chemical and physical structure of the soil is altered drastically. On remoistening the soil, large amounts of easily decomposable substances such as amino acids and proteins become available. The activity of several groups of microorganisms is enhanced, resulting in a rush of decomposition, demonstrated by an increase in CO_2-production many times larger than the natural fluctuations.

In addition the soil structure preferably should remain undisturbed. This being impossible, we tried to pretreat the soil in such a way that in all experiments its structure was nearly the same. This structure did not differ much from those under field conditions in this arable soil.

One could claim that ideal similarity between experimental and natural conditions would demand that in our experiments the temperature should fluctuate. But since it was expected that this would complicate the interpretation of the results, we started our investigations working at a strictly constant temperature.

The experimental conditions also differed from the natural ones by substituting for the gasphase of the soil with air. Moreover, the carbon dioxide tension was zero and in closed reaction vessels the oxygen content decreased considerably. However, control experiments

have shown that the mineralisation yield of amino acids was equally high in the presence of CO_2 and in its absence. In our experiments the oxygen content never dropped below a value of 10 %. As may be seen from the literature (BARTHOLOMEV and AMER, 1951), under these conditions the oxygen tension is not a limiting factor.

In a soil, stored for long periods under constant temperature and moisture conditions, the micro flora undergoes alterations. This was demonstrated by a decrease in soil respiration, as well as in an inhibiting effect on the nitrification rate of the ammonia. However, the yield of the ammonification process was not influenced (4.4).

The quantity of substrate added had to correspond to the quantity expected during a normal decay of the organic material under field conditions. Yearly approximately about 110 kg N/ha was supplied to the soil under investigation; one third being organic nitrogen (0.3 mg N/20 g of soil). This fraction consists mainly of proteinous material, which is gradually hydrolysed, resulting in a slow release of amino acids. Assuming that this quantity of 0.3 mg N/20 g of soil was normally supplied within a period of 6 months, and that it consists of amino acids, in a ten days period we had to supply 0.016 mg amino acid-N/20 g of soil.

Besides we have to account for a mineralisation of native organic matter. From measurements summarised in Table 3, it can be derived that, in a 10 days experiment 0.07 mg amino acid-N would be formed by this process assuming that the initial substrate for this process consists mainly of proteins.

Thus the total supply of amino acids in 10 days will be approximately 0.09 mg N/20 g of soil. If each amino acid accounts for about 5 % of the total amount, in a ten day experiment, 4.5 μg amino-N of this specified amino acid should be added.

To obtain reliable results we had to supply in our experiments 45 μg amino-N (0.003 mmol), roughly ten times the amount produced in the soil under natural conditions.

In section 4.5 we studied the influence of the concentration of an amino acid on its conversion. With low concentrations a relatively small amount of the amino acid-N was mineralised, whereas the relative carbon mineralisation was not affected. Therefore in our experiments the immobilisation yield of nitrogen was estimated too low; natural conditions were more favourable for humus formation.

There was also the possibility that secondary reactions might occur, disturb the results. At a high pH-value, volatilisation of ammonia can occur (JEWITT, 1942); with a low pH-value, nitrite and ammonia can react to yield nitrogen (ALLISON c.s., 1948, 1951, 1952, WYLER and DELWICHE, 1954); depending on aeration and supply of organic material, denitrification processes of different character may occur (HAUCK c.s., 1956, JANSSON c.s., 1952, BREMNER c.s., 1954, BROADBENT, 1951, PINCK c.s., 1950, WHEELER c.s., 1958, WYLER c.s., 1954, GREENWOOD c.s., 1960): and finally ammonia can be bound tightly in the lattice layers of clay minerals (ALLISON c.s., 1953, STEVENSON, 1957). These losses can be observed only under specified conditions.

Often the presence of relevant microorganisms is essential. In several experiments we have drawn up the mineralisation balance: 98–100 % of the nitrogen added was recovered.

One could claim that this total recovery could be caused by an enhanced nitrogen mineralisation of the soil organic matter, balancing one of the losses mentioned. This can be verified in tracer experiments. As may be seen from Table 3, the N-mineralisation of the native organic matter in this type of soil proceeds very slowly. Therefore it is appropriate to assume that interferences of this kind did not occur.

Putnam and Schmidt (1959) investigated the adsorption of amino acids by soil adsorption complexes. Arginine and lysine remained adsorbed after percolating with water, whereas tryptophan dissolved. Greenwood and Lees (1960) could demonstrate the adsorption of arginine, histidine, lysine and tryptophan. According to the authors mentioned the adsorbed amino acids can be attacked by microbes.

Amino acids may also disappear by incorporation in bacterial matter. This activity may be promoted when mixtures of amino acids are added in the relative amounts present in proteins. We have investigated this point by incubating soil samples with single amino acids. After 1–5 days both a watery extract and an acid hydrolysate were prepared. The amount of an amino acid was estimated semi-quantitatively after paperchromatography. It appeared that the amino acid content of the extract equalled that of the hydrolysate, demonstrating that no amino acid incorporation had taken place. At most 5 % of the amino acids added (alanine or tyrosine) could have escaped our attention through variance in the samples and in the analytical procedure.

In section 5.2. we concluded that keto acids are converted more rapidly than the corresponding amino acids. The presence of keto acids in the soil during amino acid breakdown could not be demonstrated (5.3.2.) (Greenwood and Lees, 1960).

A comparison of the relative mineralisation yields of amino acids of keto acids administered in equivalent amounts (Table 9) revealed that the keto acids had been oxidised to a slightly lesser extent than the amino acids. Although these differences were small, they were significant. It is difficult to reconcile the assumption that amino acids in soil are broken down via keto acids with these results. However, one has to consider that during amino acid breakdown, keto acid will be produced at a relatively small rate, accumulating in very small amounts, less than one percent. In the experiments with keto acids their total amount was administered at once, resulting in a hundred fold higher concentration.

For this reason we investigated the effect of the concentration of a keto acid on its relative mineralisation. For pyruvic acid and α-ketogluric acid no concentration effect could be demonstrated, their mineralisation yield appeared to be constant 62 resp. 58 %. For the corresponding amino acids these values were 71 resp. 72 %, demonstrating the dissimilarity mentioned above. Only with p-hydroxyphenylpyruvic acid the mineralisation yield decreased when the

concentration was enhanced. 16 % of this acid was mineralised when 0.1 mmol had been administered, whereas this increased to 35 % with 0.025 mmol. The relevant amino acid tyrosine was mineralised for about 67 % (Table 6). We are willing to believe that the concentration effect observed supports the assumption that the dissimilarity between the mineralisation yield of a keto acid and its corresponding amino acid may be due to the totally different amounts of keto acids present in these experiments. One has to consider that the keto acid concentrations present in the soil are less than 4 % of the amounts investigated.

Table 9 shows that ammonia added together with a keto acid enhances the carbon immobilisation somewhat.

When we consider the relative N-mineralisation yield of an amino acid in relation to its C/N-ratio it appears that the yield decreases with increasing C/N-ratio (Fig. 4a, b, c, Table 7). The dependence may be seen from Fig. 6, in which the nitrogen immobilisation is plotted against the carbon immobilisation. The C/N-ratio of the immobilised fraction is practically constant.

These experiments demonstrated, that the N-mineralisation of tyrosine was relatively larger than that of the other amino acids, including phenylalanine. For the C-mineralisation, no distinct relation seems to exist. (Fig. 5). However the C/N-ratio of the immobilised fraction was nearly equal to that of the various amino acids.

In this respect it is remarkable that a similar value of the C/N-ratio was found by WINSOR and POLLARD (1956) and by JENSEN (1932) who incubated in soil substrates as sucrose and ammonium sulfate, dried fungi, bacteria and actinomycetes. The C/N-ratio of the immobilised fraction did not alter within 90 days.

From these experiments the conclusion may be drawn that the amino acids are altered by their immobilisation. The results of the hydrolysis experiments described on page 230 and 242 demonstrated however that no direct incorporation of amino acids occurred in significant amounts. The assumption that a complete mineralisation of amino acids preceeds, followed by a direct synthesis of humic substances from their degradation products, has to be excluded.

The equality of the C/N-ratios of the immobilised fractions suggested that the same endproduct is formed independent of the amino acids administered. This can best be understood by assuming that this is caused by a synthesis of microbial matter, particularly of proteins. However, since the total amount of microbial matter in the soil is very small as compared with the total soil organic matter, it has to be rapidly synthesised and their proteins and amino acids incorporated quickly into humous products.

The finding of KORTLEVEN c.s. (1960), that under favourable conditions up to 25 % of the total supplied organic material is converted into stable organic matter supports this view. It is also in accordance with the view of SWABY (1959) that the essential stage in the immobilisation of an amino acid falls in a period shortly after the death of the cells of an organism. In this situation both quinones and amino acids

are released and are then able to react with each other. Indeed these complexes can be formed in vitro; they are fairly resistent against microbial attack. Moreover, on acid hydrolysis the amino acids are liberated, as happens on hydrolysis of humic substances. If this reaction could be shown to account for the combined amino acid fraction in soil, then the variations and differences in amino acid content of different soil samples would be easily understood.

The results obtained may be of some value for further investigations into humus formation. Special attention has to be drawn to the interaction of various compounds in soil. It seems appropriate to extend these investigations to mixtures of amino acids, both without and in the presence of phenolic substances.

The formation of complexes between amino acids and soil organic matter would involve the production of compounds with a C/N-ratio higher than that of the parent amino acids, whereas the production of protein-clay complexes will lead to complexes with unaltered C/N-ratios. Moreover, in the humic substances, polymers of non-nitrogen-containing compounds are present. The important role of lignin, polyuronides and of polyphenols is well known. However, the processes are more complicated, since external conditions exert a considerable influence on the accumulation of metabolic products essential for humus synthesis (Enders, 1943).

SUMMARY

1. In studying the origin of nitrogen in humus formation it might be valuable to consider the conversions of amino acids in soil.

2. A survey was made of the literature on the presence and on the conversions of soil amino compounds. Speculations on the character of the resistant soil amino acid fraction have been given.

3. The soil was incubated with amino acids and conversions of amino acids were studied respirometrically. Oxygen consumption and carbon dioxide evolution were determined, and the content of amino acids, keto acids, ammonia and nitrate in aqueous or 1 N sodium chloride extracts.

4. The amino acids disappeared rapidly, mineralisation being completed within a few days. The ratio of the mineralisation products remained fairly constant during the period of investigation. When aeration was sufficient, small differences in moisture content gave no rise to alterations in the mineralisation yields. Under similar conditions the nitrification was enhanced by increasing moisture content. Storing the soil sample in the laboratory under constant temperature and moisture content did not give rise to differences in the mineralisation yield, but to a marked depression in the rate of nitrification. The relative carbon mineralisation yield was independent of the concentrations of amino acid administered; the relative nitrogen mineralisation yield increased with enhancing concentration. Amino acids with a high C/N-ratio give rise to lower nitrogen mineralisation percentages than those with low values.

5. During the decomposition of amino acids in soil no significant amounts of keto acids could be detected. The keto acids were broken down more rapidly than the corresponding amino acids. Their carbon mineralisation percentages were slightly lower than with amino acids. This might be due to a concentration effect, as the keto acids in the experiments mentioned had a 100 times higher concentration. It is quite reasonable to assume that keto acids are intermediates in amino acid breakdown in soil.

6. The differences between the laboratory conditions and those in nature, and the effect on the results of the experiments are discussed. No losses of substrates or

of reaction products occur by denitrification, volatilisation of ammonia, nor by irreversible fixation of ammonia and of amino acids to soil colloids. The amino acids are deaminated oxydatively; the nonmineralised fraction has a remarkably constant C/N-ratio. This accounts for most of the amino acids investigated. The assumption of SWABY that the amino acids in organisms that had just died would combine with quinones also becoming available may explain most of the properties of the resistant soil amino acid fraction.

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NOTES ON WILD SPECIES OF MUSA FROM SUMATRA

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Our knowledge of the wild banana species occurring in Sumatra is still very scanty. Most herbarium collections are imcomplete, and but few botanist have paid attention to them in the field.

The earlier records, field observations and collections were made by the Italian botanist Beccari about eighty years ago. He described one of his findings as *Musa sumatrana*; this species was hitherto known only from the type collection. I could study the type locality of this banana, the waterfall in the Anei canyon (called Air Mantjur in Minangkebau language), West Sumatra. It grows there together with two other wild banana species which were also found by me in other parts of West Sumatra. One of them appeared to be new to science.

The study of the wild bananas occurring in Malaysia got a new impulse by the recent breeding experiments and by the taxonomic and genetical studies that were carried out by Cheesman (1947, 1948, 1950) and Simmonds (1956, 1959) at the Imperial College of Tropical Agriculture, Trinidad. I wish to thank Mr. N. W. Simmonds for his stimulating interest in my studies of the bananas occurring in West Sumatra and for his comments on my new species. My stay in Sumatra was unfortunately too short to allow an extensive exploration of its banana flora. Besides the species described below I collected on the East Coast near Pematang Siantar a form of *Musa acuminata* Colla. (Meijer 7264), which proved to agree with the so-called Cameron form of *Musa acuminata* (= *M. truncata* Ridl.) occurring in Malaya (Simmonds 1955).

Description of species

SECTION CALLIMUSA

Musa salaccensis Zollinger—Pisang karok
Backer, Handboek Flora van Java 3: 133, 1924.

Stems usually about 2–2.5 m high. Leaves small as compared with *M. sumatrana* and *M. halabanensis*. Petiole circ. 75–95 cm with violet pigmentation. Blade 250–300 cm long and 45–60 cm wide; base unequal, rounded or decurrent in the petiole; apex rounded with an about 8 cm long cirrus; underside green, not waxy; main nerve (in cross section) 20 mm high and 15 mm wide; channel 8 mm deep and 10 mm wide; secondary nerves at distances of circ. 7–12 mm; marginal nerve violet. Inflorescence erect; stalk and axis violet, somewhat rough. Peduncle circ. 20–35 cm long. Female bunch with

about ten hands; each hand with 4–5 fruits in one row. Pedicel gradually thickened towards the fruit, circ. 2 cm long. Fruits pointing upwards, very light green with violet spots, with sharp angles, 8–17.5 cm long, 12–23 mm in diameter; apex blunt; at cross section about 5 seeds visible in the white pulp; seeds turbinate, at the base circ. 5 mm broad, 6 mm long near base and halfway with ringlike furrows; colour reddish brown at ripeness. Male bud with a light red-violet colour, circ. 15 cm high and 5–6 cm in diameter. Apex of bracts green; male flowers about four in the axil of each bract, light green; compound tepal light green at upper half, greenish white at base, about 60 × 7 mm, with a sharp keel, at the apex with two lateral lobes of 4 mm, each with a hairlike apex of 2 mm and about three median lobes of 3 mm length; simple tepal 45–47 mm long, up to 11 mm wide. Stamens with white, towards the top widened, filaments, circ. 13 mm long, flat, up to 2 mm wide at apex; anthers faintly yellowish white, (26–30) × 3 mm, outwards curved at base. Ovary 4 mm long.

Specimens examined:

SUMATRA, West Sumatra, Ophir district, G. Talamau, 800 m, Bünnemeyer 509; Pajakumbuh region, Mt. Sago, margin of forest, 900–1000 m alt., Meijer 5788 (L). Also noted near Air Mantjur in the Anei canyon, W. Sumatra.

On Mt. Sago, W. Sumatra, this species is the most common wild banana at an altitude of about 900–1200 m. It occurs also in Java (BACKER, loc. cit.). The name "pisang karok" is also used in Malaya, though for a different species. The male bud has a bitter taste, and is not considered to be a good vegetable. The fibers are rather good for binding purposes. CHEESMAN (1950) considered *Musa violascens* Ridl. from Malaya to be a narrowly related but distinct species. The fruits of *M. violascens* are biseriate according to SIMMONDS (1955) in stead of uniseriate as in *M. salaccensis*. I could confirm this during a trip in Malaya, 1960.

SECTION MUSA

Musa sumatrana Beccari ex André, Illustr. Hortic. 27, 37. t. 375, 1880 —Pisang palapak.

Plants about 3–4 m high. Petioles 40–60 cm or longer (90–100); edges of fissures and underside as well as the sheath violet coloured. Blade 200–300 cm long and 50–72 cm broad; base more or less cordate with rounded lobes resembling auricles; apex blunt-rounded; cirrus deciduous; main nerve at cross section about 11 mm thick and 12 mm wide; upper side with channel of circ. 4 mm depth and 7 mm width; side nerves at distances of 6–8 mm; marginal nerve violet; underside of leaves glaucous green, waxy. Inflorescences bent down; stalk of bunch circ. 30–35 cm long, green, glabrous; axis of inflorescence green, somewhat hairy; fruiting bunch circ. 50–70 (100) cm long and 15–25 cm in diameter, usually with circ. 15–18, but in luxuriously growing plants with many more hands. Fruits circ. 15–24 per hand, biseriate, often negatively geotropic; base of

pedicels at 14–17 mm from the axis; pedicel 15–17 mm, rather abruptly passing into the fruit, which is 7–15 cm long and 1.2 cm in diameter, lightgreen, more or less terete with only one faint keel; apex rather acute, 5–7 mm long. Distance between the hands on the axis of the inflorescence circ. 5 cm; place of insertion of the hands about $\frac{1}{2}$ of that of the axis.

Seeds embedded in white pulp, circ. 5 visible in a cross section, flat like the seeds of *Solanum melongena*, 5–7 mm in diameter, enveloped by a thin aril adhering with the central part in the form of a ring-like hilum 1.5 mm in diameter; margin sharp but irregular in outline; at centre 2 mm thick, black at ripeness. Male bud on stalk of 35–50 cm; on fruiting plants rather oblong turbinate, about three times as long as wide (8–14 × 2.5–5 cm). Bracts with a dark violet-red colour, also at the inside. Flowers in two rows in the axil of the bract, seven to eight per row. Compound tepal circ. 25 mm long, translucent, except at the yellow apex, which consists of a row of three blunt lobes of 3 × 2 mm and two inner ones of 2 × 1 mm; simple tepal 7 mm long, 5 mm wide, apex acute, serrulate; filaments and anthers dirty white; filaments terete, circ. 13 mm long, 1 mm diam.; anthers 12 mm long, at the base not curved outwards, together 1.5 mm wide; ovary 3–4 mm long.

Specimens examined:

SUMATRA, West Sumatra, Air Mantjur, Anei canyon, alt. circ. 360 m, Beccari, Piante Sum. 489, August 1878 (Type, isotype herb. Kew); same locality, Meijer 7334 (herb. L, cult Hort. Bot. Leiden); Pajakumbuh region, Mt. Sago, alt. circ. 900 m, Meijer 5786, 5787, 5809 (L).

This species is narrowly related to *Musa acuminata*, especially to ssp. *microcarpa* (Becc.)Simmonds from Borneo. Future, more extensive studies will have to consider whether its status is that of a species or of a subspecies. The seeds, flat and with a sharp rim provide the best diagnostic characters. They were described in the original description as follows: "semina valde compressa margine ancipiti irregulariter dentata" (Ill. Hort. 27: 37. 1880). *Musa halabanensis* Meijer differs in its larger dimensions, the broader male bud, the lighter colour of the innerside of the bracts found in the male bud and in the shape of its seeds, which are not flat and much smaller.

Musa halabanensis Meijer sp. nov. — Pisang tjirik minjak.

Planta robusta. Caulis 9 m altus. Lamina 6 m longa et 0.9 m lata. Flores feminei circ. 2.5 cm longi. Fructus teretes, 6–7.5 cm longi, apice acuminati. Semina globosum, 4 mm diam. Inflorescentia pars mascula ante anthesin conoidea; bracteae extus violaceae, intus ochraceae. Flos masculus circ. 5 cm longus.

Plants large; stems up to circ. 9 m high, at the base with diameter of 20–23 cm. Leaf sheaths and base of stalk dark violet brown; the juice of all parts of these plants is turning into a brown elastic resin called "tjirik minjak" which in the Menangkabau language means dirty oil. Petiole circ. 60–100 cm long, with two sharp edges. Blade 2.3–5 m long, 60–90 cm wide; base truncate, often unequal; apex

bluntly rounded with a cirrus of 4–8 cm; underside light green coated with a thin layer of wax. Main nerve green, at the underside with a slight pigmentation, in cross section 23 mm high and 20 mm wide, with a 12 mm wide and up to 9 mm deep channel. Distances between side nerves 13–17 mm; margin of the leaf violet. Peduncle circ. 40–60 cm long, glabrous or softly hairy; young inflorescences with green inner bracts of 70 × 9 cm, which when the inflorescence is detached turn light violet; outer bracts circ. 27 × 8 cm, with recurved apex. Female flowers with a white translucent simple tepal, which is rather wide-boatshaped but without a large keel, 2.5 cm long, 1 cm wide from keel to margin, dented near the apex, i.e. near the apical lobe of 1.5 × 1.5 mm. Compound tepal with yellow lobes, the rest dirty white, with 2 keels; lateral lobes 4 mm wide at base, 6 mm long. Stamens without well-developed anthers, sometimes with one anther only; circ. 2 cm long; style circ. 22 mm long; stigma brownish black, sticky, circ. 4 mm broad and 3.5 mm long. Bunch of fruits with about 8–9 hands; distances between the hands circ. 4–5 cm; fruits biseriate, 9–12 (in another plant 13–19) in each row; base of pedicels at 7–8 mm from axis of inflorescence, pedicel not sharply set off from the fruit, 2–3 mm wide, circ. 1–1.5 cm long. Fruit 6–7.5 cm long, at first faintly keeled, at ripeness terete, curved, negatively geotropic or ageotropic; colour light yellow-green, abruptly narrowed into the 12–13 mm long apex which is darker green than the fruit itself; the skin at ripeness splitting from apex to base into valves and showing a white pulp with glistening black, globular-pyramidal seeds (about 10 visible in a cross section of an unripe fruit), with a small white hilum of 0.5 mm diameter at point of attachment and with a flat underside, 2–3 mm high, 3–4 mm broad.

Stalk of male bud circ. 60 cm in fruiting plants; male bud ovoid, shouldered near the base, circ. 12 × 20 cm in one case and 9 × 15 cm in another, with dark violet bracts; inner side of bracts yellow ochre, with circ. 40 nerves; in the axil of each of these bracts two rows of 12–14 male flowers; the latter in a bud of medium age about 5 cm length; compund tepal circ. 35 mm long, at apex with ochre-yellow lobes, 2 of them pointing inwards and 3 outwards, each of them circ. 2 mm wide and 3 mm long; the two lateral lobes moreover curved sidewards; the two inner curved towards the medium part of the tepal; simple tepal translucent, 17 mm long, 7 mm wide, with blunt minutely serrulate circ. 1–1.5 cm long apex.

Stamens circ. 34–35 mm long; anthers (22–24) × 2 mm, yellowish white; filaments flat, 1.5 mm wide at apex; base of anthers in some stamens at different heights, not curved inwards (as in *M. salaccensis*); ovary circ. 5 mm high.

Specimens examined:

SUMATRA, West Sumatra, Mt. Singgalang, Beccari P. Sum. 148; Anei canyon, alt. 300 m, Meijer 7533 (L); Pajakumbuh, Mt. Sago near tea garden Halaban, alt. circ. 800–900 m, Meijer 5785 (L), 5789 (type, L), 5808 (L); 7463 (L, also cult. in Hort. Bot. Leiden).

Mr N. W. Simmonds (in lit.) remarked with regard to this species

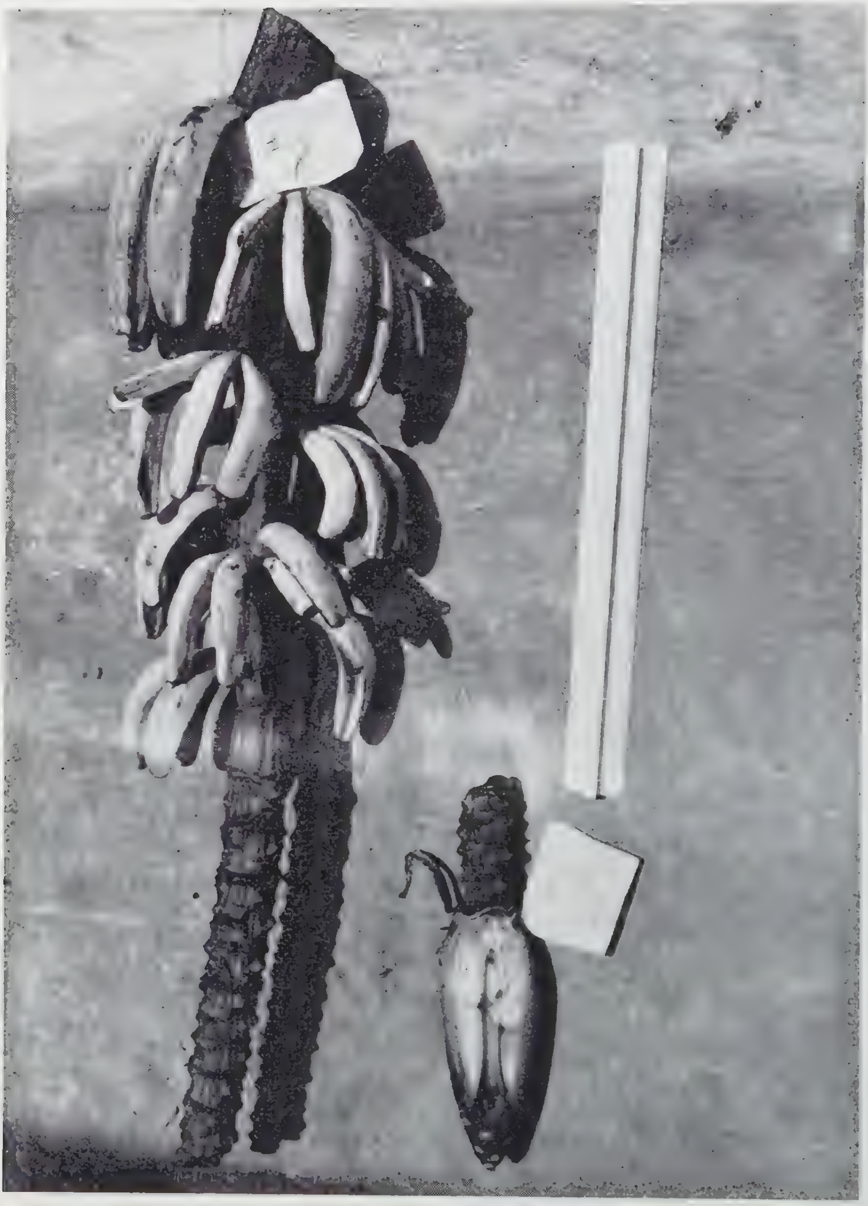

Plate 1. *Musa salaccensis* Zollinger. Pisang karok. Male bud and fruiting bunch. coll. Meijer 5788.

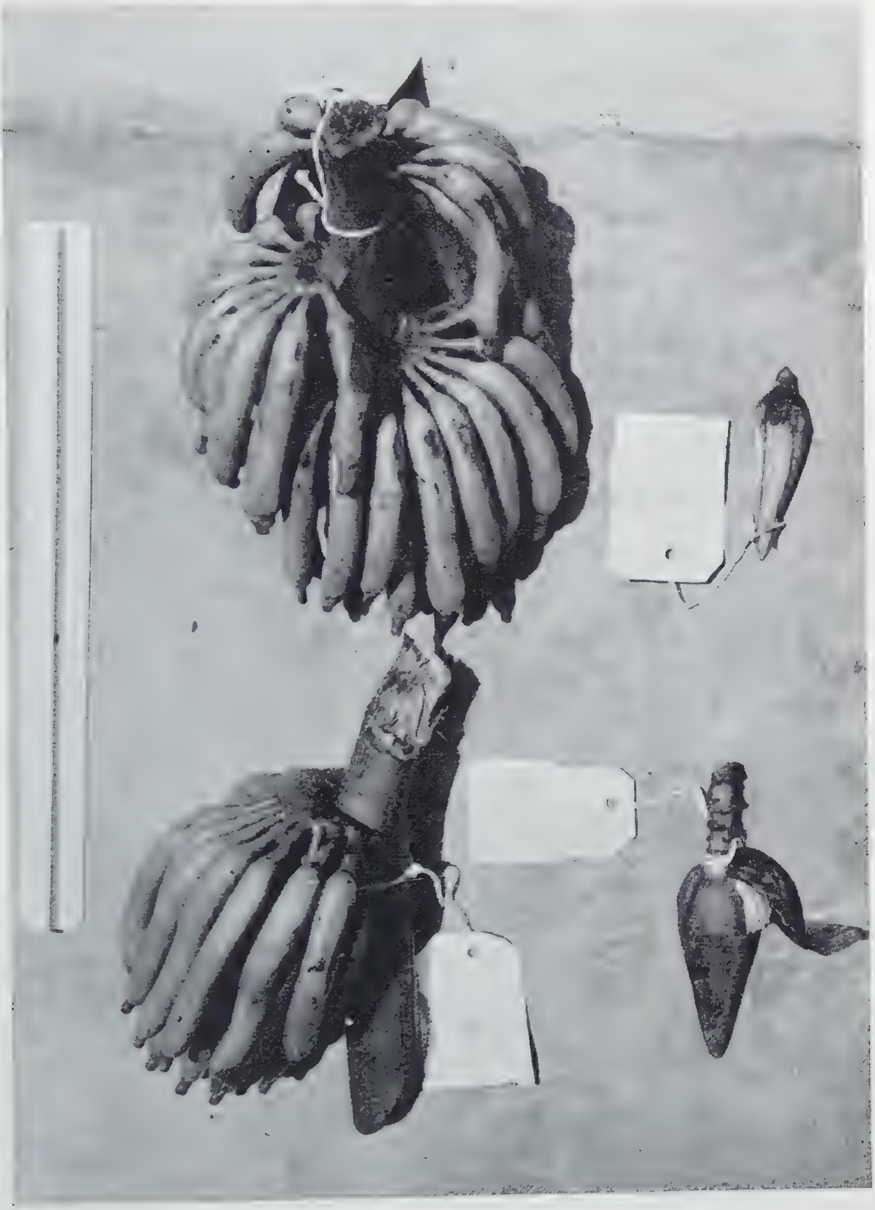

Plate 2. *Musa sumatrana* Beccari – Pisang palapak. Fruits and one male bud from Meijer 5787; male bud with bracts and flowers visible from Meijer 5786.

Plate 3. *Musa halabanensis* Meijer, nov. spec. Male bud with stalk and fruits on axis of inflorescence from Meijer 5789 (type); other fruits and male bud: Meijer 5785. Maesure stick 30 cm.

Plate 4. *Musa sumatrana* Becc. – Pisang palapak. Halaban, G. Sago, West Sumatra, 700–800 m alt.

that its very small seeds are quite unlike those of any well understood banana species. Moreover, in herb. Calcutta and herb. Bogor, he has seen seeds from Amboina, referred to *Musa sylvestris* Rumph. The chromosomes were counted at Trinidad by Mr. K. Shepherd, and found to be 22. For this reason the plant is certainly to be referred to *Musa* section *Musa* (see also Simmonds, 1960). According to my assistant Maradjo, who showed this wild banana to me in the forests of W. Sumatra, the male bud of *M. halabanensis* is a good vegetable. The large dimensions of all parts of the plant, the abundant, very sticky juice, the large diameter of the male bud, the shape of the fruit and the small globular seeds are a sufficient justification for regarding it as a new species.

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MARSILEALES AND SALVINIALES — "LIVING FOSSILS"?

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(*received May 2nd, 1961*)

According to current opinion (see, e.g., Christensen 1938, Reed 1954, Zimmermann 1959, Pichi-Sermolli 1959) there is no close relationship between the Marsileales and the Salvineales, so that they should not be united into one class as "Hydropteridales". Their taxonomic position with regard to the *Filices Leptosporangiatae*, to which class they have up to now usually been referred, has always been unsatisfactory. Not only has a close relationship with any group of real ferns never been demonstrated, but their heterospory—to my mind an extremely fundamental character—and the absence of a true annulus distinguish them sharply from the Leptosporangiate ferns.

The resemblance between certain fossil "seeds", later identified as fructifications of the *Caytoniales* (Thomas 1925, 1927), and the sporocarps of *Marsilea* has in the past lead to the interpretation of these Caytonialian fructifications and the associated *Sagenopteris* leaves as remains of *Marsileaceae*. When Thomas recognised these remains as those of advanced Pteridosperms, this interpretation was abandoned. However, Zimmermann (1930) again suggested that *Marsileales* might be descendants of Caytonialian stock in the same way as *Isoëtes* is a dwarfed "survivor" of the *Lepidodendrales*. Thomas and Harris (1951) critisized this idea and mentioned several differences between the two groups under discussion. Indeed it is not very likely that *Marsilea*, which is undoubtedly more primitive than the *Caytoniales* in several respects, could have descended from the latter. Among other things, the *Caytoniales* produced unisexual strobili and seed-like fructifications, whereas *Marsilea* has bisexual sporocarps which are shed before fertilisation takes place and do not produce "seeds", the embryo developing at once into a young plant.

On the other hand I agree with Zimmermann that the differences mentioned by Thomas and by Harris are not so important as to preclude any relationship. However, the interpretation of a possible relationship must be based on an entirely different assumption, *viz.*, that instead of *Marsilea* being a form descended from the Caytonialian ancestors, the *Caytoniales* descended from a group of more primitive plants of which the *Marsileaceae* are the survivors. I am personally convinced of the fact that the basic ancestral stock of the *Caytoniales*, the *Cycadophyta* and ultimately of the Angiosperms is to be sought among the *Glossopteridales*. Consequently, the above-mentioned suggested derivation of the *Caytoniales* from Marsileaceous ancestors

would imply that the *Marsileales* represent surviving members of the large Pteridospermous class of the *Glossopteridales*. Indeed, the *Marsileaceae* show several agreements with seed ferns of this type. Bisexual reproductive organs were described from *Glossopteris* (s.l.) by PLUMSTEAD (1956) and, barring the *Bennettitales* and the Angiosperms, no other group of the *Pteropsida* shows this singular character, except the *Marsileales*. The general habit of the *Marsileales*, *i.e.*, a rhizomatous stem producing fronds of which the fertile ones bear in the basal region one to several *stalked* sporangium-bearing organs, is known from several *Glossopteridales* (Plumstead, see also HARRIS 1958). The frond segments of *Marsilea* resemble those of the common form-genus *Sagenopteris* and possess a type of venation which is "glossopteroid". Recently, cells resembling sieve-tube members have been found in *Marsilea* (WHITE 1961), an advanced condition unknown in ferns. The structure of the megasporangium resembles that of gymnospermous types, especially those of the *Cycadales* and the female gametophyte is as much reduced as one would expect in a seed fern. The multiciliate spermatozoids are of a type suggesting a relation with those of the *Cycadales* but they are obviously more primitive than the latter, because they uncoil before fertilisation whereas the spermatozoids of the Cycads have a spiral band of cilia which is fused with the protoplasmic "body". Neither of these characters alone is sufficiently convincing, but the complex of similarities is thought to be fairly significant. I am, therefore, of the opinion that at least one may accept the above-mentioned suggestion as an alternative hypothesis concerning the relationships of the *Marsileales*—the *Marsileaceae* do not show any clear affinities to the truc ferns whereas there are some, though admittedly slender indications of a relationship with Glossopterid seed ferns and higher Gymnosperms. As far as I can ascertain there are no arguments strongly pleading against this assumption and, therefore, I am inclined to regard the *Marsileaceae* as surviving members of the *Glossopteridales*.

Similarly, the *Salviniales* can be interpreted as descendants of the *Lyginopteridales* (*Lagenostomales*). *Salvinia* produces rhachis-borne "sporocarps" and is markedly heterosporous. Its vegetative parts are built like those of certain pinnate types of Pteridospermous fronds; the similarity between the "leaflets" (i.e., the pinnae!) of *Salvinia* and the pinnae of *Neuropteris* is rather striking. The structure of the megaspore of the Salviniaceae is so strongly reminiscent of that of the *Lagenostomales* (see Fig. 1), that this is, to my mind, not likely to be a mere coincidence. The "perisporium", then, would be the homologue of a pteridospermous cupule, which has become reduced to a unicellular layer as a result of the adaptation to an aquatic habitat. Significantly, the perisporium develops as an outgrowth of the megasporangiophore and gradually envelops the megaspore. It is still provided with an apical pore in *Azolla*. Current opinion among pteridologists is that the *Salviniales* must be divided into two families, the *Salviniaceae* and the *Azollaceae*. Indeed, the sporangia are borne on the frond in the *Azollaceae*, but this is a condition known to have

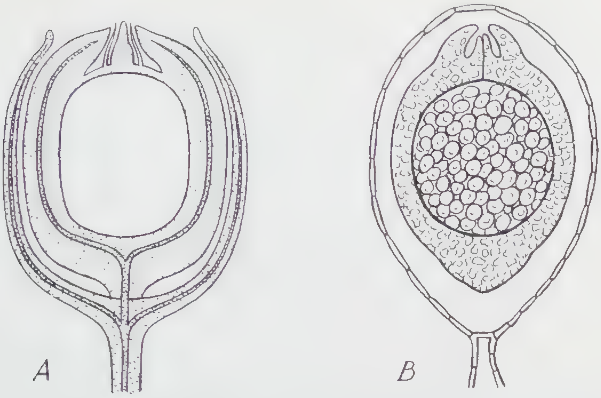

Fig. 1. A, Cupulated megasporangium of *Lagenostoma* and B. mature megasporangium of *Salvinia*, both in optical longitudinal section.

occurred in several types of seed ferns such as *Pecopteris*. Rather recently, REMY (1953) described a fossil type of reproductive organ (*Saarotheca sphenopteroides*, see Fig. 2) which had not previously been encountered. This organ was attached to a sphenopteroid frond and Remy suggested as one of several tentative interpretations that this fossil might be related to the water ferns. I think that this discovery provides some additional evidence of the relationship between the *Salviniales* and the *Lyginopteridales*, for *Saarotheca* might be interpreted as an ancestral form of the male "sporocarp" of the *Salviniales*.

The eventual transfer of the *Marsileaceae* to the *Glossopteridales* and of the *Salviniaceae* and *Azollaceae* to the *Lyginopteridales* would allow us to consider the features of the living plants to be (necessarily rough) approximations of the respective conditions in the related classes of seed ferns thought to be long extinct. It is, for instance, not at all improbable that at some later date, when fossilized Glossopterid fertile organs with retained anatomical structure become available for study, the morphology of the sporocarps of the Marsileaceae might

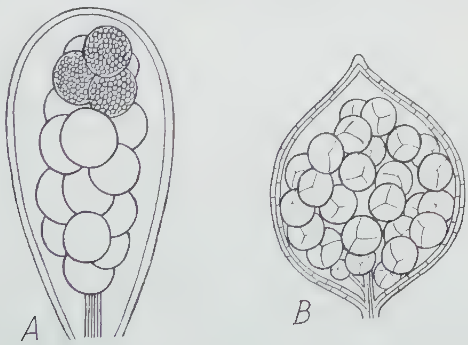

Fig. 2. A, *Saarotheca sphenopteroides* Remy, reproductive organ, and B. male sporocarp of *Azolla filiculoides*.

provide a clue to the interpretation of the structural features of the corresponding fertile organs of this interesting group of fossil plants. Conversely, the discovery of more fossil material may eventually supply more conclusive evidence in favour of the alternative hypothesis regarding the taxonomic relationships of the water ferns and that is why the idea is tentatively suggested here, the main purpose of the suggestion being to invite criticism.

Even if they are considered to be derived from certain groups of Pteridosperms, the survival of the water ferns is not so surprising as it might seem to be. *Isoëtes* and the recently discovered *Stylites*, descended from equally ancient ancestral stock, also occur in an aquatic or semi-aquatic habitat. The mode of sexual reproduction of these "living fossils" and of the *Hydropteridales* depends on the presence of free water and only in an aquatic habitat is their reproduction not at a great disadvantage in respect of the very efficient method of sexual reproduction of the Angiosperms, so that they can still successfully compete, undoubtedly assisted by their vegetative reproduction, whereas the terrestrial seed ferns already long ago were ousted out by the competitive pressure of the more efficiently reproducing seed-forming plants (*Cycadophyta* and *Angiospermae*).

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DIFFUSION AND ABSORPTION OF IONS IN PLANT TISSUE

I. OBSERVATIONS ON THE ABSORPTION OF AMMONIUM BY CUT DISCS OF POTATO TUBER AS COMPARED TO MAIZE ROOTS

the late T. H. VAN DEN HONERT and J. J. M. HOOYMANS [1])

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(*received April 29th, 1961*)

1. Introduction

Since the early studies of Steward, cut discs of potato tuber tissue have been used widely for experiments in the field of ion absorption. In the laboratory of the authors they have been used during the winter months especially when it has been difficult to grow maize plants of sufficient absorption capacity.

Striking differences became apparent in the behaviour of potato discs and maize with respect to the absorption of NH_4-ions under different conditions of concentration, pH and presence of other cations.

There is some evidence that the phenomena observed in potato discs are not restricted to that object, which does not function as an ion absorbing organ under normal conditions, but also occur in the roots of some plant species, and thus are of a more general importance for our understanding of the process of ion absorption.

2. Material and methods

a. Plant material

Maize plants (single cross D $\times$ 9) were grown in essentially the same way as described in an earlier paper (cf Van den Honert, Hooymans and Volkers 1955).

Discs of potato tuber tissue (commercial strain "Bintje") were prepared according to the procedure introduced by Steward (1930). In short, tissue cylinders with a diameter of 24 mm were cut with a cork borer and sliced with a slicer of simple construction to discs with a thickness of 2 mm. A hole with a diameter of 2.5 mm was cut in the centre of each disc with another cork borer.

After cutting the discs were strung on a knitting-needle each disc being separated from its neighbour by a porcelain bead. Each needle carried 30 discs.

The discs were rinsed in flowing tap water during 4 days before being used in the experiments. Between experiments they were placed again in flowing tap water. In this way they usually stayed healthy

[1]) The text of this paper was written by us as much according to Van den Honerts latest concepts as possible. Nevertheless we hold ourselves responsible for the contents. G. G. J. Bange and J. J. M. Hooymans.

for about 14 days during which time about 10 experiments were performed. Sets of discs that had lost their turgescence or caused some opalescence in the solution were discarded.

b. Experimental vessels

Maize plants were kept in cylindrical beakers the content of which varied from 250 to 1500 ml according to root development.

The experiments with potato discs were performed in cylindrical tubes with a diameter of 28 mm and a length of about 350 mm. The volume of the experimental solution in each tube was 120 ml throughout. At the bottom the tubes narrowed and here the air supply was connected.

Both beakers and tubes were aerated continuously during the experiments, the aeration served also for the purpose of stirring.

c. Maintenance of temperature and pH

The experimental vessels were kept in a waterbath maintained at $20 \pm 0.1°$ C. During the experiment the pH was checked frequently and, if necessary, adjusted in the way described earlier (cf Van den Honert, Hooymans and Volkers 1955, page 146). With freshly cut discs the pH usually remained constant but with older discs it tended to shift to the acid side.

d. Experimental procedure

Different plants or different sets of discs may vary considerably in their absorption capacity. To compensate for this, essentially the same technique as described in earlier papers was used (cf Van den Honert 1933 and Van den Honert and Hooymans 1955). Standard or unity uptake was defined as the rate of NH_4-absorption from a 0.5 me/l $(NH_4)_2SO_4$-solution in the presence of a modified Woodford and Gregory culture solution (see table 1) at a pH of 6.0 and a temperature of 20° C. Standard rates of uptake were determined for each plant or set of 30 discs before and in some cases after an experiment.

Table 1

Composition of the culture solution

K_2SO_4	0.277 me/l
KH_2PO_4	0.151 me/l
$CaSO_4$	0.204 me/l
$MgSO_4$	0.195 me/l

Transfer of plant tissue from one solution to another involves rapid initial equilibrations. Where steady state absorption was the subject of study all tissues were allowed an adjustment period of at least 30 minutes in a solution of the same composition as was to be used in the experiment proper.

The time during which the tissues were allowed to absorb ammonium from the solutions depended on the actual rate of uptake. Too large a decrease in concentration of the experimental solutions

had to be avoided, and too small a decrease enlarged the experimental error. On the average the decrease amounted to 20 to 30 %. However, at high concentrations it had to be smaller to avoid too prolonged absorption periods.

The range between the concentration at the beginning and at the end of an experiment has been indicated by horizontal lines in Figs 1, 2, 3, 4 and 8.

e. NH_4-estimation

Ammonium was estimated according to the method described by ALLPORT (1947).

All concentrations are expressed in milliequivalents per litre (me/l).

3. RESULTS

a. NH_4-absorption as related to NH_4-concentration

In maize the relation between the rate of NH_4-absorption and NH_4-concentration in the presence of the culture solution (Fig. 1) has the characteristics of a Langmuir adsorption isotherm the absorption being largely independent of concentration at concentrations higher than 0.6 me/l. A halfvalue of about 0.11 me/l can be computed.

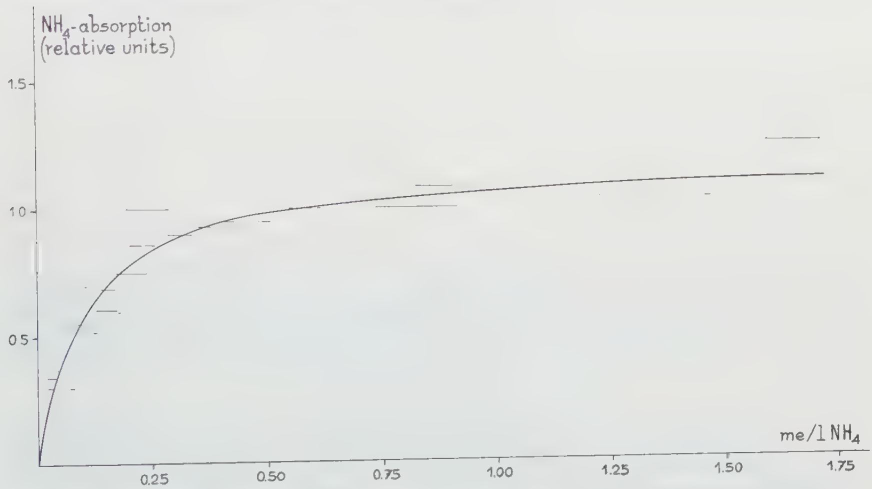

Fig. 1. Relation between NH_4-concentration and rate of NH_4-absorption by maize plants in the presence of a dilute culture solution (pH = 6.0, t = 20° C). ------ = standard uptake.

In striking contrast NH_4-absorption in potato discs in the presence of the culture solution (Fig. 2, A and B) does not attain a maximal value at so low NH_4-concentrations. Here a relatively steep initial rise in the concentration range from 0 to about 0.1 me/l grades into a slower rise at higher concentrations but a maximum is not reached until a concentration of about 30 me/l. The shape of the curve deviates from the simple Langmuir adsorption isotherm.

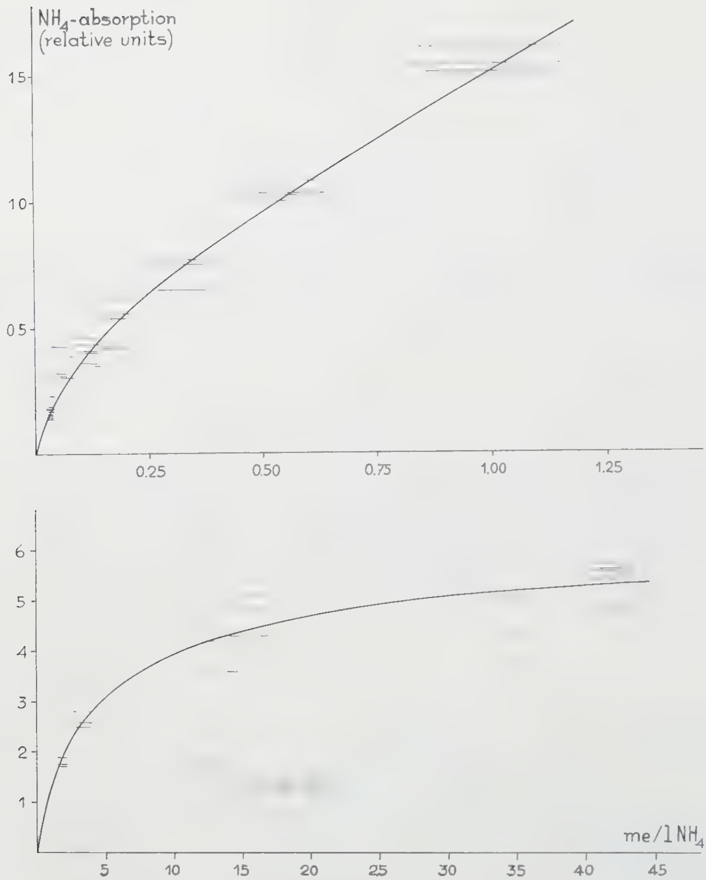

Fig. 2. Relation between NH_4-concentration and rate of NH_4-absorption by potato discs in the presence of a dilute culture solution (pH = 6.0, t = 20° C). A. Concentration range from 0 to 1.2 me/l NH_4. B. Idem from 0 to 44 me/l NH_4. ------ = standard uptake.

In the absence of the culture solution the qualitative features of the uptake remain essentially the same (Fig. 3, A and B). However, the rate of absorption in the concentration range from 0 to about 10 me/l is higher than in the presence of the culture solution.

If the culture solution is replaced by a solution containing 10 me/l $CaCl_2$ + 10 me/l KCl, the same steep initial part is observed again (Fig. 4, A) but thereafter the curve rises more slowly and no maximum is attained within the range of concentrations used (Fig. 4, B).

b. Influence of other cations on NH_4-absorption

In maize the addition of increasing amounts of Ca (as $CaCl_2$) to a 0.5 me/l $(NH_4)_2SO_4$-solution affects the rate of NH_4-absorption to a much less extent than in potato discs as may appear from a comparison of Figs. 5 and 6.

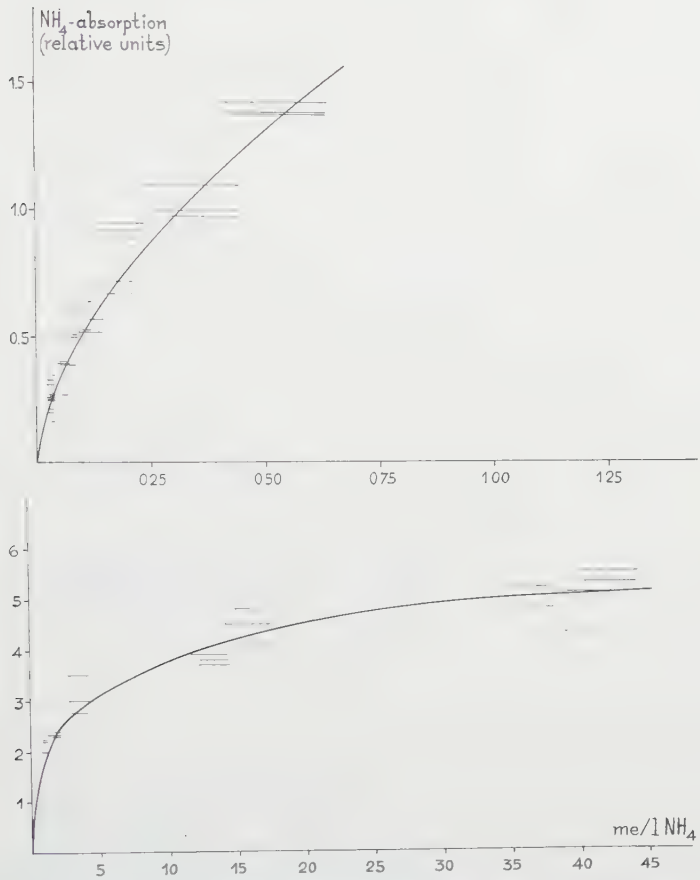

Fig. 3. Relation between NH_4-concentration and rate of NH_4-absorption by potato discs in the absence of other cations (pH = 6.0, t = 20° C). A. Concentration range from 0 to 0.7 me/l NH_4. B. Idem from 0 to 44 me/l NH_4.

The effectiveness of other mono- and polyvalent cations in reducing NH_4-absorption in potato discs appears to increase in the order Li = Na < K < Ca < La. It should be noted that apparently the rate of NH_4-absorption does not fall below a certain level even with excess cation as is especially clear in the case of La.

c. Influence of pH on NH_4-absorption

The influence of pH on the rate of NH_4-absorption in maize and in potato discs shows some striking resemblances on the one hand and striking differences on the other.

In maize there is hardly any influence in the pH-range from 4.3 to 6.0 (Fig. 7). However, an increasing stimulation of NH_4-absorption is observed at pH-values above 6.0. The amount of the increase at a certain pH-value shows a linear relationship with the

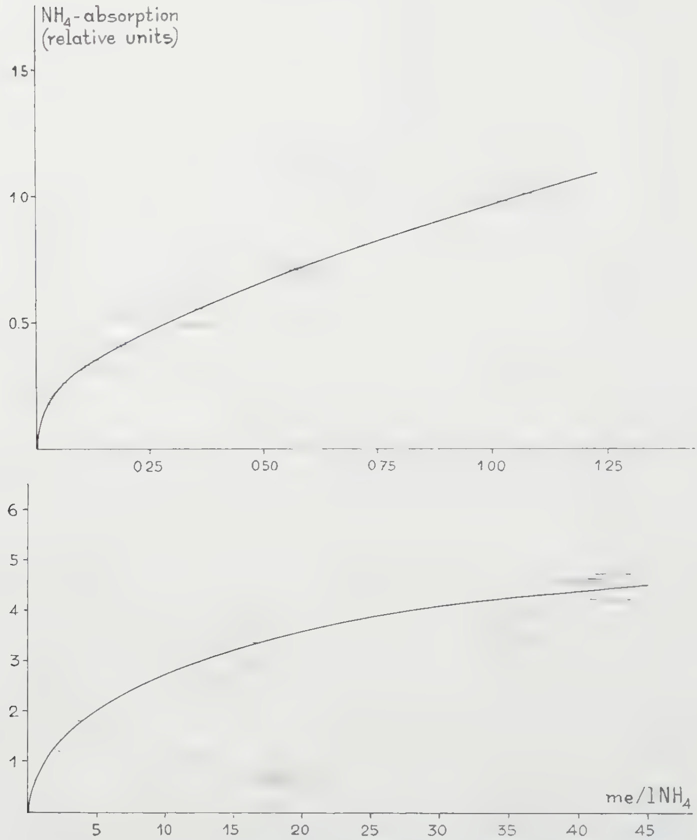

Fig. 4. Relation between NH_4-concentration and rate of NH_4-absorption by potato discs in the presence of 10 me/l KCl + 10 me/l $CaCl_2$ (pH = 6.0, t = 20° C). A. Concentration range from 0 to 1.2 me/l NH_4. B. Idem from 0 to 44 me/l NH_4.

NH_4-concentration used. This is demonstrated by the fact that a straight line is obtained when the relations between NH_4-concentration and NH_4-absorption at pH = 6.0 and pH = 7.4 are subtracted (Fig. 8, dotted line).

The same behaviour with respect to pH-values above 6.0 is shown by potato discs irrespective of the presence of other salts in the $(NH_4)_2SO_4$-solution (Fig. 9).

In contrast, the behaviour of potato discs at pH-values below 6.0 strongly depends upon the composition of the solution (Fig. 9). If nothing is added to the $(NH_4)_2SO_4$-solution there is a marked fall in the rate of NH_4-absorption between pH = 6.0 and pH = 4.0. This fall is completely checked by the presence of 10 me/l KCl + 5 me/l $CaCl_2$, the culture solution taking an intermediate position. In all cases, about the same level of NH_4-absorption is attained at pH = 4.0.

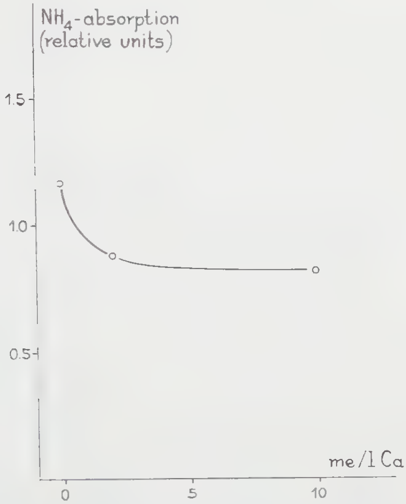

Fig. 5. Relation between the rate of NH_4-absorption by maize plants from a 0.5 me/l NH_4-solution and the concentration of Ca added as $CaCl_2$ (pH = 6.0, t = 20° C).

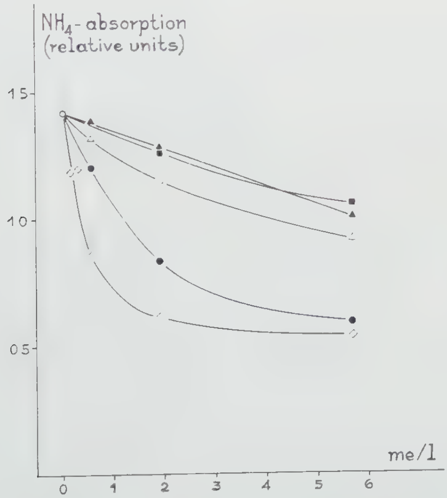

Fig. 6. Relation between the rate of NH_4-absorption by potato discs from a 0.5 me/l NH_4-solution and the concentration of mono-, di- and trivalent cations added as their chlorides (pH = 6.0, t = 20° C).

■ = Li ● = Ca
▲ = Na ◇ = La
△ = K

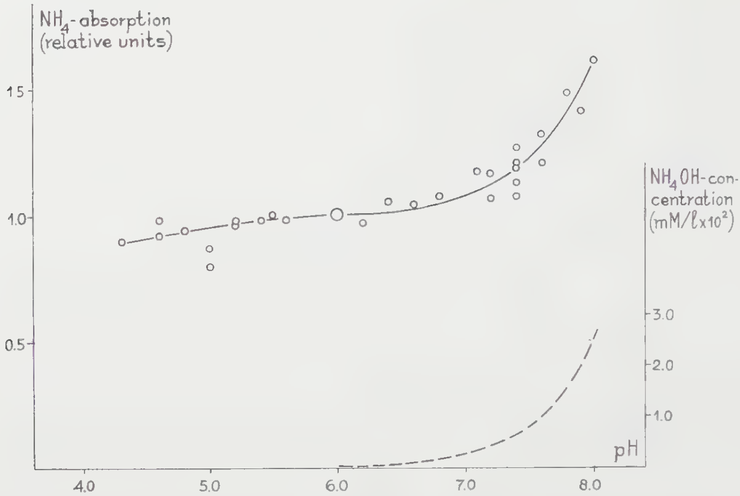

Fig. 7. Relation between the rate of NH_4-absorption by maize plants from a 0.5 me/l NH_4-solution and pH in the presence of a dilute culture solution ($t = 20°$ C). The dotted line represents the amount of undissociated NH_4OH in the solution.

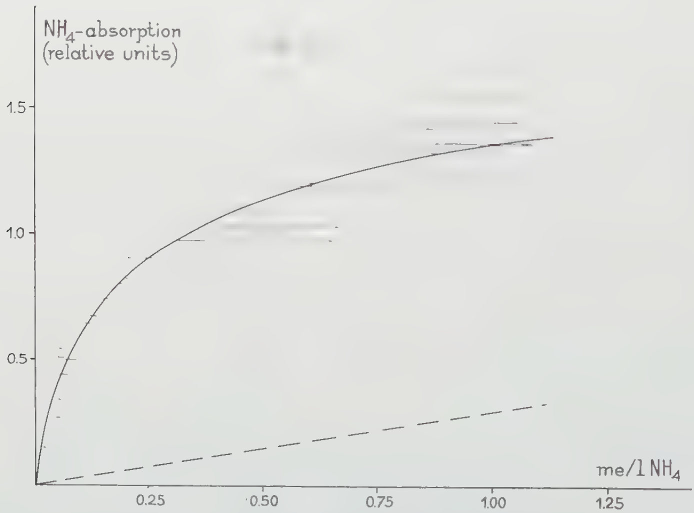

Fig. 8. Relation between NH_4-concentration and rate of NH_4-absorption by maize plants at pH = 7.4 in the presence of a dilute culture solution. The dotted line represents the difference between the rate of uptake at pH = 7.4 and at pH = 6.0 (as derived from Fig. 1).

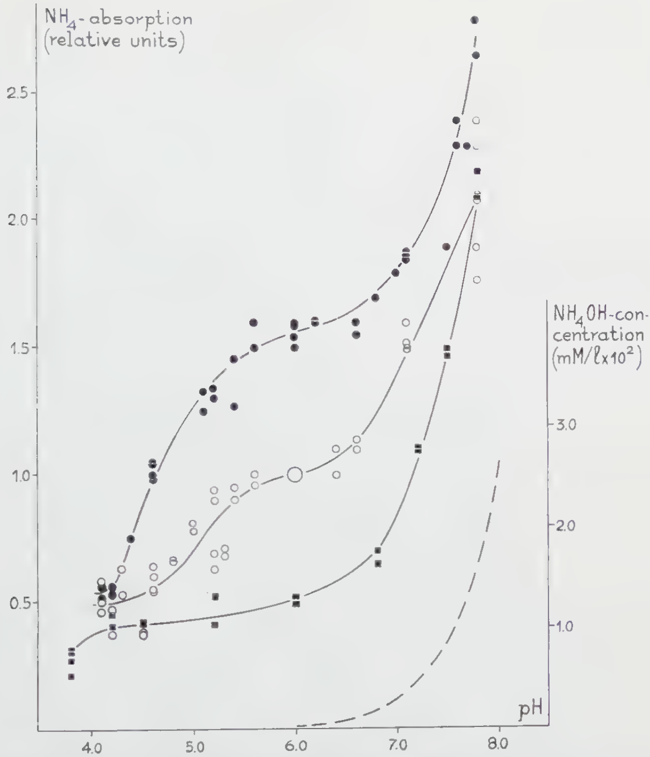

Fig. 9. Relation between the rate of NH_4-absorption by potato discs from a 0.5 me/l NH_4-solution and pH ($t = 20°$ C). The dotted line represents the amount of undissociated NH_4OH in the solution.

● = no other cations present
○ = in the presence of a dilute culture solution
■ = in the presence of 10 me/l KCl + 5 me/l $CaCl_2$.

4. Discussion

It is tempting to attribute the marked differences in the characteristics of NH_4-absorption by the two objects to secondary complications in the absorption process rather than to differences in the primary absorption mechanism. These secondary complications conceivably are related to questions of supply and removal, a problem that has been given extensive treatment by Briggs and Robertson (1948).

Therefore the following hypothesis is advanced to explain the experimental results.

In potato discs the ratio between the rate of passive equilibration of the free space with the environment and the rate of active NH_4-absorption by the protoplasts is such that the NH_4-ions are removed too rapidly from the free space to allow its equilibration with the surrounding solution. In this case a steady state of absorption will be established for the disc as a whole in which a gradient of NH_4-con-

centration in the free space is maintained from the outside to the inside of the disc.

The hypothesis implies that NH_4-absorption in this material is not restricted to the outer surface but also occurs in at least part of the inner protoplasmatic surfaces lining the free space.

On the other hand in maize either the capacity to absorb ions is largely or entirely restricted to the outer surface of the root or the ratio between the rate of passive equilibration of the free space with the environment and the rate of active NH_4-absorption by the protoplasts is quite different from the ratio in potato discs. In case of a large ratio the cortex cells will be embedded in a solution differing not markedly from the outward solution. A concentration gradient of any importance will occur only at the lowest concentration. On the other hand a small ratio will prevent the NH_4-ions from penetrating to any extent into the bulk of the free space, so that at low external concentrations the uptake will be almost completely limited to the outer surface of the root. Only at much higher concentrations will the internal uptake become relatively important.

What differences in the behaviour of the two objects are to be expected on these assumptions?

In maize as soon as the concentration of the ion in the solution has reached the level at which the absorption mechanism is saturated, the rate of absorption for the tissue as a whole will become relatively independent of concentration, the effect being due either to a saturation of all cortex cells or of the outer surface of the root only (Fig. 1).

On the other hand in potato tuber tissue, when the concentration in the solution has attained the level of carrier saturation it will be only the outer surface of the disc that is able to absorb at its maximal rate. All inner surfaces will still be operating at a lower level. Only when the concentration rises further will the region of carrier saturation extend gradually from the outside to the inside of the disc until at last all inner surfaces absorb at their maximal rate. So the absorption curve will not become level after its initial steep rise but will go on rising with a gradually decreasing slope until for the disc as a whole saturation is attained (Figs. 2, 3 and 4).

The presence of a large number of fixed negative charges has been demonstrated for the free space of potato tuber tissue (BANGE 1957). Consequently diffusion will proceed at least partly in an ionic exchange region (I.E.R.) and for this reason will show special features such as interaction with other cations and pH-effects.

The addition of mono- or polyvalent cations to the NH_4-solution will lower the NH_4-concentration in the I.E.R. and so reduce its diffusion pressure. Therefore in potato discs the rate of NH_4-absorption will rise more rapidly with concentration in the absence of other cations and more slowly in the presence of a high K + Ca-concentration than in the culture solution (Figs 2, 3 and 4). At high NH_4-concentrations, however, the difference due to the absence or presence of the culture solution will vanish owing to its relatively low concentration (Figs 2 and 3). On the other hand in the presence of a high

concentration of K + Ca the NH_4-concentration at which for the disc as a whole saturation is attained, will move to higher values because the bivalent ion especially will largely eliminate any adsorption of the NH_4-ion. Actually in this case the saturation level was not attained within the range of concentrations used (Fig. 4).

In accordance with the principles of ion exchange the effectiveness of an added cation in reducing the diffusion pressure and so the absorption of the NH_4-ion will depend on its charge. Monovalent ions such as Li, Na and K will be less effective than a bivalent ion such as Ca which again will be inferior to the trivalent La-ion (Fig. 6). The level of NH_4-absorption in the presence of excess cation will represent the situation in which for the NH_4-ion all adsorption is eliminated and, as far as NH_4-diffusion is concerned, the whole free space is Water Free Space.

In maize roots NH_4-absorption will be affected by Ca to a much less extent (Fig. 5) either because the uptake is hardly limited by diffusion or because that part of absorption limited by diffusion is only a small fraction of the total uptake.

An effect of cations on NH_4-absorption by potato discs was described by ASPREY as early as 1933 and explained in terms of ion antagonism.

A pH-effect on diffusion in the I.E.R. and thus indirectly on NH_4-absorption is to be expected in the pH-range where the degree of dissociation of the acid groups is reduced. Actually there is a steep decline in the rate of NH_4-absorption between pH = 6.0 and pH = 4.0 when no other cations are present (Fig. 9). It is obvious that the decline will be smaller in the culture solution and practically absent in a solution with a high K + Ca-content, the diffusion pressure of the NH_4-ion in these cases having been reduced already by the addition of cations.

In maize no such pH-effects will occur in the pH-range between 6.0 and 4.3 (Fig. 7).

The peculiar pH-effect at pH-values higher than 6.0 common to maize roots and potato discs (Figs 7 and 9) is supposed to stand in no relation to the diffusion phenomena described above but is tentatively explained in the following way.

With rising pH increasing amounts of undissociated NH_4OH will be present in solutions of NH_4-salts. Molecular NH_4OH is known to penetrate readily through the permeation barriers of the cell. The assumption therefore is that the effect is due to the diffusion of NH_4OH into the cells in which it is subsequently fixed by a process which is not rate-limiting.

This hypothesis is supported by two observations.

In the first place if it is assumed that the absorption of the NH_4-ion is independent of pH at pH-values higher than 6.0, all absorption above the level of pH = 6.0 appears to be proportional to the amount of NH_4OH present in the solution. This amount is indicated by the dotted lines in Figs 7 and 9.

In the second place it was demonstrated for maize that the extra

absorption at pH = 7.4 is proportional to the concentration of the NH_4-salts in the solution (Fig. 8, dotted line) as the hypothesis requires.

It should be noted that a similar phenomenon was observed by HURD and SUTCLIFFE (1957) and by HURD (1958, 1959) in K-absorption from KCl-solutions by different kinds of storage tissue and by barley roots. The authors relate the effect to the increasing availibility of HCO_3-ions at higher pH-values. Their statement that the phenomenon was not observed in potato discs makes a common explanation, however tempting, less likely. It is true that in the experiments described above $(NH_4)_2SO_4$ and not NH_4Cl was used but the stimulation of the absorption by alkaline pH-values was not affected by the presence of excess Cl-ions (Fig. 9).

To return to the main subject under discussion, there is another question that deserves attention. A close inspection of the curves of Figs 3A and 4A reveals that absorption at the lowest concentrations is hardly affected by the presence of a high K + Ca-content in the solution. Moreover absorption in the interior of the disc only would not show the sharp changes in slope of the curve of Fig. 4A but rise more smoothly from the origin. The obvious explanation is that at the low concentrations an absorption component not mediated by cation sensitive diffusion becomes relatively important and that this component is identical with the absorption at the outer surface of the disc.

A rough estimation of the contribution of the outward surface to the total absorption capacity of the disc can be made by a tentative extrapolation of the slowly rising portion of the curve of Fig. 4A to the ordinate. This procedure yields a value of about 0.25. The total absorption capacity of the disc being about 5.2 the share of the outward surface can amount to not more than 5 %. In case of cells of a cubical shape this would mean that about 3 superficial layers would participate in the uptake provided the absorption capacity were evenly distributed over the surface of the absorbing cells. However, this is likely to be an underestimate because the absorption capacity may be supposed to decrease less abruptly to the interior of the disc.

Do the experiments described above contain any clue to the chemical nature of the fixed negative charges in the diffusion medium?

It was shown (Fig. 6) that the efficacy of added cations in reducing the rate of NH_4-absorption increases in the series

$$Li = Na < K < Ca < La.$$

Comparison of this sequence with the ionspectra for sulphate, phosphate and carboxyl colloids (BOOY and BUNGENBERG DE JONG 1956) suggests that carboxyl rather than one of the other types of colloids are involved.

This conclusion is supported by the strong decrease in the rate of NH_4-absorption between pH = 6.0 and pH = 4.0 (Fig. 9). Apparently the dissociation constant (pK) of the acidic groups is higher than 4.0, but due to the complexity of the system a satisfactory estimation from these indirect data is not possible. At any rate, the

estimation seems too high for the much stronger acidic sulphate and phosphate groups.

Therefore a tentative identification of the dissociable groups with carboxyl groups of the cell wall pectin seems justified. This conclusion is in agreement with other evidence (LATIES 1959, page 94–95) though the discrepancy with the results of BANGE (1957, cf LATIES l.c.) remains a point to be elucidated.

SUMMARY

NH_4-absorption by maize plants and by cut discs of potato tuber was studied in relation to NH_4-concentration, pH and concentration of added mono- and polyvalent cations.

Striking differences in the behaviour of the two objects became apparent.

The results are tentatively explained on the assumption that during steady state absorption in potato discs a gradient of NH_4-concentration is maintained in the free space.

Several explanations for the different behaviour of maize roots are discussed.

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DIFFUSION AND ABSORPTION OF IONS IN PLANT TISSUE

II. TIME COURSE OF NH_4-ABSORPTION BY CUT DISCS OF POTATO TUBER AT DIFFERENT TEMPERATURES

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1. Introduction

Though the phenomena described in part I are readily explained by the hypothesis put forward, the authors realize that several other explanations might be advanced.

In the first place, where no data are presented to prove the contrary the rates of absorption measured and supposed to represent active absorption might still contain a passive component not saturated during the prerinsing period. In other words a steady state might not yet have been established during this period and consequently the free space (W.F.S. + I.E.R.) might still have continued to equilibrate with the external solution during the experimental period.

In the second place, two binding sites may be involved in NH_4-transportation, a specific one transporting NH_4 only and having a high affinity for this ion and a general cation binding site transporting mono- as well as di- and trivalent cations but as far as NH_4 is concerned with a much lower affinity than the specific site. A general cation carrier has been described for the yeast cell by Conway and Duggan (1956).

However, a close examination of the data renders the second explanation less likely. It appears from Fig. 6 (part I) that the addition of excess cation reduces the rate of NH_4-absorption to a level not lower than about 0.5 of the standard value. In terms of competition for a general cation carrier this would mean that all NH_4-absorption at this point proceeds by means of the specific site. So the relation between the rate of absorption and the concentration of NH_4 in the presence of excess cation in the range of low NH_4-concentrations should be of the Langmuir type. From Fig. 4A (part I) it is clear that even in the presence of 10 me/l Ca + 10 me/l K this is not the case.

To exclude the first possibility as well as to collect more direct experimental evidence for the existence of a concentration gradient in the free space of the disc during steady state absorption at 20° C, a series of experiments was performed in which the time course of NH_4-absorption was studied at 20° C as well as at 0° C, i.e. under conditions of checked active absorption. These experiments will be described in the following sections.

2. Material and methods

The technique used was essentially the same as described in part I. The diameter of the discs was 17 mm. To enlarge the decrease in concentration and to enable the measurement of absorption during periods as short as a few minutes, 60 instead of 30 discs were strung on one needle. The volume of the experimental solutions in each tube amounted to 75 ml.

All discs were first rinsed in flowing tap water for 4 days. Following this, the discs were immersed in tap water at 0° C for several hours. Then the experiment at 0° C was started with one half of the discs, meanwhile the other half was immersed in tap water at 20° C for $1\frac{1}{2}$ hours before starting the experiment at 20° C.

Before starting each experiment, the discs were given three 10 minute rinses in distilled water of the experimental temperature and then, after being blotted by a gentle rolling of the needles over dry filter paper, they were transferred to the experimental solution.

$(NH_4)_2HPO_4$-solutions buffered at pH = 6.0 with tris in a concentration of 170.8 mg/l were used. The use of this solution does not change the essential features of the absorption-concentration relation as discussed in part I.

NH_4-absorption was measured by estimation of the decrease in concentration of the experimental solution. Without proper precautions this procedure would have led to a larger decrease in concentration and consequently a smaller mean concentration the longer the absorption period. Therefore one or more mls of a concentrated $(NH_4)_2HPO_4$-solution were added to the tubes in the experiments at 20° C when the duration of the absorption period was one hour or longer. In this way variations in mean concentration between the tubes were kept well within 12 %.

An experiment in which the release of NH_4 was studied was performed in the following way. Three needles with 60 discs each, pretreated in the way described previously, were allowed to absorb NH_4 at 0° C in three tubes containing a 0.556 me/l $(NH_4)_2HPO_4$-solution. After 150 minutes the needles were blotted and transferred to three tubes containing a 10 me/l $CaCl_2$-solution of the same temperature. This solution was renewed at increasing intervals and its NH_4-content measured.

NH_4 was estimated in the way described in part I.

3. Results

The relation between the amount of NH_4 absorbed and time at 0° C and at 20° C was studied for two NH_4-concentrations, viz 0.556 and 2.222 me/l. The experiments were performed in duplicate and their results averaged. They are represented in Figs 1 and 2 respectively.

At both concentrations and at 20° C as well as at 0° C there appears to be a very rapid initial phase of absorption completed in 4 minutes or less. At 0° C this rapid initial phase passes into a phase with a

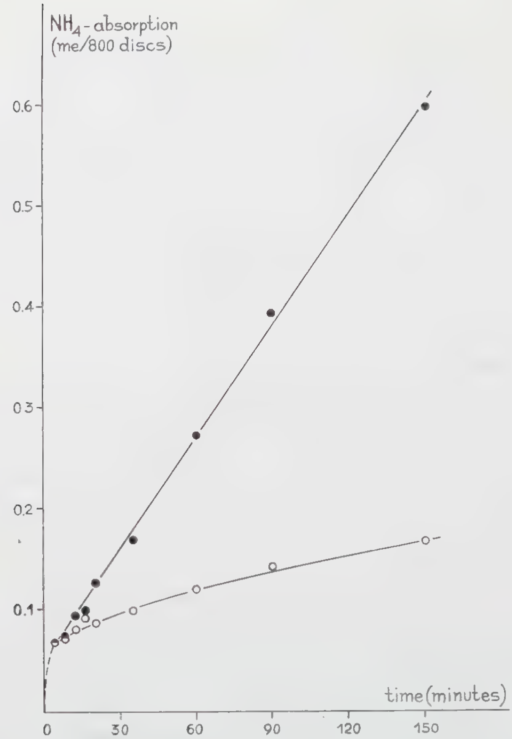

Fig. 1. Time course of NH_4-absorption at 0° C (open circles) and at 20° C (full circles) from a 0.556 me/l NH_4-solution at pH = 6.0 (mean of two experiments)

slower and gradually decreasing rate of absorption over a period of at least 150 minutes. However, at 20° C a steady rate of absorption is reached immediately after the rapid initial phase and is maintained over a period of at least 150 minutes.

In order to examine whether all NH_4-absorption at 0° C is essentially passive and consequently reversible, an experiment was performed in which the release of NH_4, after its preliminary absorption over a period of 150 minutes from a 0.556 me/l NH_4-solution at 0° C, was studied. The results are presented in Fig. 3. Again a very rapid initial phase of loss precedes a phase of slower and gradually decreasing NH_4-release. After 8 hours all NH_4 absorbed has been recovered.

4. Discussion

The experiments described show that a prerinsing period of 30 minutes as used in the experiments of part I amply suffices to reach steady state conditions. At the same time they provide conclusive evidence for the existence of a concentration gradient inside the discs during active absorption under the prevailing conditions as will be clear from the following considerations.

At 0° C it takes 150 minutes or more for the free space of the discs

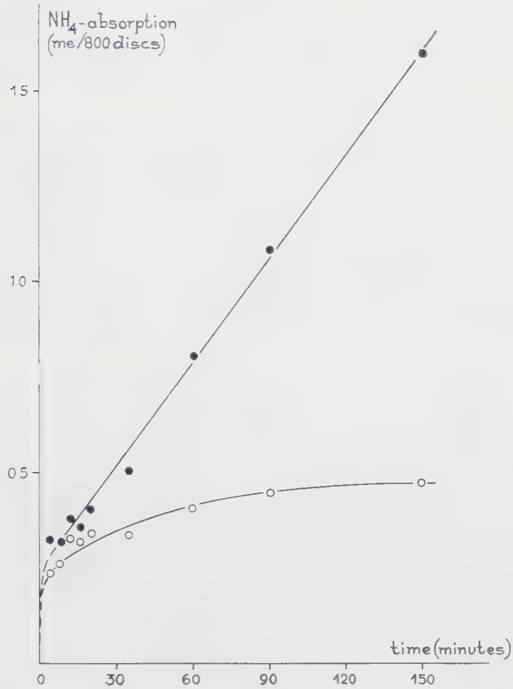

Fig. 2. Time course of NH_4-absorption at 0° C (open circles) and at 20° C (full circles) from a 2.222 me/l NH_4-solution at pH = 6.0 (mean of two experiments).

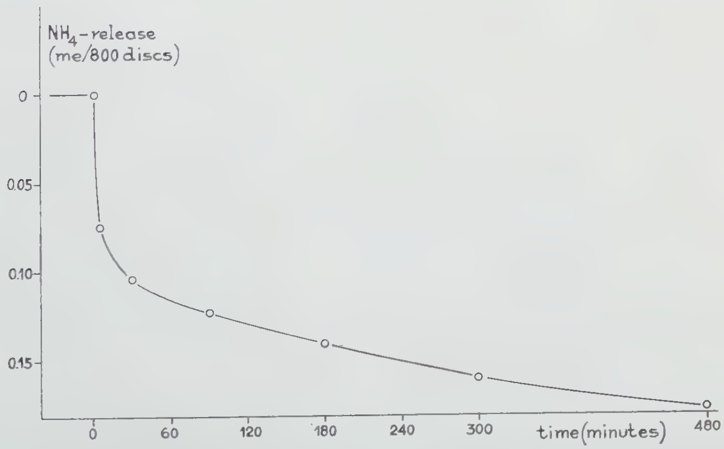

Fig. 3. Time course of NH_4-release to a 10 me/l $CaCl_2$-solution at 0° C and pH = 6.0 after absorption of 0.18 me NH_4 per 100 discs during a period of 150 minutes from a 0.556 me/l NH_4-solution at 0° C and pH = 6.0.

to equilibrate with the external solutions (Figs 1 and 2). The passive nature of the absorption process at this temperature is demonstrated by the fact that all NH_4 absorbed in 150 minutes is released again to a 10 me/l $CaCl_2$-solution (Fig. 3).

On the contrary, at 20° C only 10 minutes or less are required for a constant rate of absorption to be established (Figs 1 and 2). Even allowing for the fact that going from 0° to 20° C NH_4-diffusion is enhanced by a factor 1.7 (at least in free water) this can only mean that at the latter temperature the free space has not equilibrated with the external solution because active removal of NH_4 from the free space by the absorbing cells will retard and not enhance the equilibration. In other words there will exist a gradient of NH_4-concentration in the free space of the discs while they are in the act of active absorption.

A rough estimation of the degree of equilibration shows that the gradient is not of minor importance. At the low concentration (Fig. 1) a steady state of absorption has been attained before the equilibration has proceeded to about 40 % and the same figure for the higher concentration (Fig. 2) is about 65 %. Still these figures must be considerable overestimated because they do not take into account the retardation of the equilibration by the active process. Moreover, the rather steep initial parts of the time curves at 0° C suggest that an important part of the free space is external or in other words consists of adhering water, a not unlikely supposition considering the technique of blotting used (cf chapter 2). A similar steep initial rise is mentioned by Briggs (1957, page 321) for slices of carrot tissue and explained in essentially the same way. This circumstance would reduce the estimates mentioned above still further.

Another aspect of the results needs attention. The ordinate intercepts of the time course extrapolates at 20° C are at both concentrations much smaller than those of the estimated asymptotes of the equilibration curves at 0° C. So interpretation of the intercepts in terms of Apparent Free Space (A.F.S.) amounts would lead to serious underestimates. As pointed out by Briggs and Robertson (1957, page 16) a similar underestimate results if metabolic absorption at the cellular level does not start at a steady rate from zero time. In such cases a close examination of the equilibration curves under conditions of checked metabolic absorption will reveal whether or not an internal concentration gradient is involved. This fact seems to have been overlooked by Kylin in considering his data (Kylin 1960, Fig. 6). Likewise it is doubtful that Higinbotham and Hanson (1955) were justified in attributing such meaning as they did to the ordinate intercepts of the absorption-time relations in their experiments on Rb-uptake by potato discs.

A slow start of metabolic NH_4-absorption at the cellular level, if occurring in our experiments, would not invalidate the arguments presented for the existence of a concentration gradient because it would lengthen and not shorten the time needed for establishment of the steady state.

The possibility of the existence of concentration gradients inside the absorbing tissue should be taken into consideration especially in all work with discs of storage tissue.

SUMMARY

The time course of NH_4-absorption by cut discs of potato tuber tissue was studied at 0° C and at 20° C.

The results point to the existence of a gradient of NH_4-concentration inside the absorbing tissue during steady state absorption at 20° C.

This work was performed as part of the programm of the Foundation for Research on Plant Nutrition at Leiden, Holland.

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QUELQUES NOUVELLES ESPÈCES DE TARAXACUM, NATIVES D'EUROPE

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(*reçu le 13 juillet 1961*)

Resumé

Les espèces suivantes sont nouvelles pour la science:

Sect. *Ceratophora* Dahlst.: *T. gallicum* v. S.: France.

Sect. *Palustria* Dahlst.: *T. turfosum* (Sz. Bip.) v.S.: Autriche, Allemagne.

Sect. *Vulgaria* Dahlst.: *T. balearicum* v.S.: Espagne; *T. congestolobum* v.S.: Suisse; *T. fasciatiforme* v.S.: Suisse; *T. iseranum* v.S.: France; *T. lucidepedatum* v.S.: Portugal, Angleterre; *T. mediterraniforme* v.S.: France; *T. ruborum* v.S.: France.

Sect. *Alpina* Hagl.: *T. authionense* v.S.: France; *T. pyropum* v.S.: France.

Sect. *Fontana* v.S. (?): *T. graiense* v.S.: France, Suisse? Autriche?

Sect. *Erythrosperma* Dahlst. em. Lb. f.: *T. lambinoni* v.S.: France; *T. pseudo-dunense* v.S.: France; *T. pseudo-proximum* v.S.: Pays-Bas; *T. retzii* v.S.: France.

Les figures, publiées ici avec l'autorisation du Directeur de l'Herbier d'Etat (Leyde), sont d'après des photographies des types-specimen, prises par M. *C. L. Marks*, à qui j'exprime volontièrement mon obligation.

SECT. CERATOPHORA DAHLSTEDT

Taraxacum gallicum van Soest **sp. nov.** (Fig. 2)

Planta humilis, 4–7 cm alta.

Folia 2–6 cm longa, gramineo-viridia intense violaceo-maculata, petiolis subalatis purpureis. Folia exteriora anguste obovata vel late lingulata obtusa, retroverso- vel patente dentata; folia interiora lobata, lobi laterales (utrinque ca. 3) ad 5 mm longae triangulares, retroversi, acuti, dorso ± dentati, interlobiis sublatis brevis; lobus terminalis indeterminato-subhastatus, subdentatus subacutus.

Scapi crassi, araneosi, sub involucro dense araneosi, floriferi foliis subaequilongi.

Involucrum crassum 15 mm longum, 15 mm latum, obscure viride. Squamae exteriores laxe adpressae, ovatae, inconspicue marginatae, obtusae, pro parte cornutae; squamae interiores corniculatae, apice violaceae.

Calathium planum radians ad 2,5 cm diametro, saturate luteum. Ligulae marginales planae, extus pro parte stria cano-purpurea notatae, summis purpureae. Antherae polliniferae; stylus et stigmata livescentia. Floret aestate. Achenium ignotum.

E sectione Ceratophorum Dahlstedt.

FRANCE (Savoie): Val d'Isère, schistes humides le long de la route montant au col d'Iseran, 2600 m environ, 17.7.1959, B. de Retz 43759 p.p.

Cette espèce est très remarquable; elle est trouvée en compagnie de *T. aequilonare* Hand.-Mazz. L'aire de la section des *Ceratophora* est arctico-alpine; dans les régions boréales et arctiques de l'Europe, de l'Asie et de l'Amérique de nombreuses espèces ont été signalées, dont plusieurs sont très fréquentes. Dans les Alpes la section se présente en 2 ou 3 espèces endémiques; *T. krätlii* v.S. est rare dans les Alpes orientales, tandis que *T. gallicum* habite les Alpes occidentales, autant qu'on sache que dans un seul endroit. *T. gallicum* diffère de toutes les autres espèces de la section par les feuilles nettement maculées de tâches violettes.

C'est un caractère presque exclusif pour la section des *Spectabilia* Dahlstedt et y présent dans un grand nombre d'espèces; l'aire géographique des *Spectabilia* s'étend de l'Europe boréale, lelong de la côte Atlantique jusqu'en Espagne septentrionale.

Dans l'évolution du genre, les *Spectabilia* semblent être dérivées des *Ceratophora* et, probablement, ont pris naissance dans une des époques interglaciaires; dans les Alpes peu de *Ceratophora* sont restées comme relics. *T. gallicum*, combinant donc certains caractères des deux sections, devient par là encore plus intéressant.

SECT. PALUSTRIA DAHLSTEDT

Taraxacum turfosum (Schultz Bipontinus) van Soest (Fig. 3)

Planta humilis 3–5 cm alta.

Folia canescenti-viridia glabra, lineari-lanceolata vel lanceolata, denticulata vel sinuoso-dentata, vel brevissime hamato-lobata, subobtusa, ad 7 cm longa petiolo angusto purpureo inclusa.

Scapi ca. 2, purpurei, araneosi, foliis subaequilongi, recurvati, apicibus adscendentibus.

Involucrum parvum, laete viride, 11 mm longum, 9 mm latum. Squamae exteriores lanceolatae ad 6 mm longae, purpurascentes inconspicue sublate marginatae, laeves, erecto-patentes apice recurvatae; squamae interiores apice fusco-violaceae.

Calathium paulo radians ad 1,5 cm diametro; ligulae breves, subpallide luteae, marginales extus $\pm$ totaliter fusco-violacea. Antherae (parce?) polliniferae; stylus et stigmata sordide lutea.

Achenium (planta non typica!) parvum ca. 3 mm longum (pyramide exclusa) superne spinulosum ceterum sublaeve, in pyramiden subcylindricam ca. 0,6 mm longam subabrupte abiens; rostrum 8 mm longum, pappus sordide albus, 5 mm longus.

E sectione Palustriorum Dahlstedt.

AUTRICHE (Autriche Supérieure): in turfosis circa Wasserlos pr. Mondsee, R. Hinterhuber, C. H. Schultz-Bipontinus, Chichoriceotheca 72 (h. L.; BM, BR, K, P), sous le nom de *T. salinum* Sz.Bip. var. *turfosa*: "*pallidum, gracile, rhyzoma breve, fibrillosum*".

La description citée, donnée par Sz. Bip., n'est pas suffisante, de sorte que j'ai donné une description plus complète ci-dessus; la des-

cription des akènes est donnée auprès du matériel de cette espèce, récolté en Allemagne (Bavière): Seeshaupt, juin 1904, W. J. Jongmans (L.)

T. turfosum est nettement différenciée de *T. suecicum* Haglund (*T. palustre* Dahlstedt) par les folioles involucrales extérieures très étroites et ne pas appliquées sur l'involucre. Dans sa Monographie du genre *Taraxacum* (1907) von Handel-Mazzetti a mentionné cet exsiccat sous le nom de *T. paludosum* (Scopoli) Schlechter, ainsi que sous les "formes intermédiaires" de *T. vulgare* (Lmk.) Schrank et *T. paludosum*, probablement à cause des caractères concernant les folioles involucrales extérieures.

SECT. VULGARIA DAHLSTEDT

Taraxacum balearicum van Soest **sp. nov.** (Fig. 4)

Planta gracilis 10–15 cm alta.

Folia suberecta canescentia parce araneosa, petiolis angustis roseis, nervo mediano pallide virido, lobata; lobi laterales (utrinque 3–4) breve triangulares, ad 5 mm longi, paulo retroversi subobtusi vel inferiores angusti acuti, integres; interlobiis 0–1,5 cm longis, pro parte angustis; lobus terminalis breve hastatus vel deltoideus, ad 1 cm longus obtusus, integer; folia interiora interdum petiolo subalato.

Scapi subaraneosi, floriferi foliis subaequilongi.

Involucrum mediocre, ca. 12 mm longum, ca. 13 mm latum, basi subrotundatum, atro-viride. Squamae exteriores laxe adpressae, lanceolatae, 7–8 mm longae, ad 2,5 mm latae, anguste purpureo-marginatae, apice violaceae, laeves. Squamae interiores apice violaceae.

Calathium paulo radians ad 2 cm diametro, luteum. Ligulae marginales planae, extus stria atro-violacea notatae. Antherae polliniferae; stylus et stigmata livescentia. Floret vere. Achenium ignotum; pappus albus.

ESPAGNE (Baléares): Mallorca, chemin à l'entrée du jardin d'Alfabia, dans les environs du col de Sóller, 2.4.1953, L. Kofler (h. R. de Litardière et h. v. Soest).

Cette espèce est rapprochée de *T. affine* Jordan, dont elle est, en outre, differentiée par les feuilles dentées et les capitules nettement étroits.

Taraxacum congestolobum van Soest **sp. nov.** (Fig. 5)

Planta sat humilis, 3–5 cm alta, araneosa, basi incrassata.

Folia numerosa, subdecumbentia, canescenti-, saepe obscure viridia, ad 13 cm longa, petiolo rufescenti-colorato inclusa. Folia interiora irrigulariter lobata lobis minoribus dentibus longisque; lobi laterales (utrinque ca. 7) lingulati, patentes vel ± retroversi vel recurvati, saepe constricti, ad 2 cm longi, acuti vel obtusi, dorso ± dentati; interlobiis 5–7 mm latis, 0–2 cm longis irrigulariter sublobatis et saepe recurvato-dentatis et denticulatis; lobus terminalis saepe sagittatus vel subhastatus, 1–2 cm longus, lobuli basis saepe

elongati, ad 15 mm longi, acuti vel obtusi, lobulo apicali saepe subconstricto, obtuso vel subobtuso. Folia exteriora minus lobata.

Scapi subnumerosi, floriferi foliis conspicue breviori.

Involucrum 15 mm longum, 15 mm latum. Squamae exteriores laxe patentes vel denique recurvatae, lanceolatae, ca. 8 mm longae, 2,5 mm latae, purpurascentes, anguste inconspicue marginatae; squamae interiores apice rufo-violaceae, omnes laeves.

Calathium subclausum. Ligulae marginales clausae vel canaliculatae vel raro angustissime planae, (subpallide) luteae, extus purpurascentes, apice pallide purpureae. Antherae parce polliniferae; stylus luteus, stigmata paulo virescentia. Floret aestate.

Achenium (planta non typica!) olivaceo-stramineum, 4 mm longum (pyramide exclusa), superne spinulosum, ceterum ± laeve, in pyramiden conico-cylindricam, 0,6 mm longam abrupte abiens; rostrum 7 mm longum; pappus albus, 5 mm longus.

Suisse (Grisons): Albula pass, 2100–2200 m, 17.7.1948, v. Soest (h 19325). Ensuite:
Valais: Saas-Fée, Plattjen, 1800 m et 2400 m; et pied du Mittaghorn, 1800 m, tous 1952, v. Soest (h); Val Ferret, 1961, v. Soest (h).
France (Savoie) Val d'Isère, Bois de Rogoney, 1900 m, de Retz (43718).

Taraxacum fasciatiforme van Soest **sp. nov.** (Fig. 6)

Planta 10–15 cm alta.

Folia obscure viridia, paulo glaucescentia, lobata, in nervo dorsali rufescento-colorato densiuscule araneosa, ca. 12 cm longa petiolo anguste alato purpureo inclusa. Folia interiora lingulata; lobi laterales (utrinque ca. 5) ad 2 cm longi, triangulares, retroversi, dorso saepe curvato-dentati vel denticulati, acuti; interlobiis ca. 5 mm latis, ad 8 mm longis, rufo-purpureis, margine plicatulis, saepe sublonge dentatis; lobi superiores saepe approximati; lobus terminalis sagittatus vel anguste et elongato-hastatus vel elongato-deltoideus, 1–3 cm longis, acutus — subobtusus, integer vel raro incisus, lobuli basis ± retroversi vel patentes, acuti.

Scapi araneosi, floriferi foliis aequilongi vel breviter superantes.

Involucrum 15 mm longum, ad 18 mm latum. Squamae exteriores lanceolatae, retroflexae, ad 10 mm longae, 2–3 mm latae, purpurascentes; squamae interiores apice fusco-violaceae, pro parte callosae.

Calathium planum radians, ad 3,5 cm diametro, saturate luteum. Ligulae marginales planae, extus stria cano-violacea notatate. Antherae polliniferae; stylus et stigmata laete lutea. Floret aestate. Achenium ignotum, pappus albus.

E sectione Vulgariorum Dahlstedt.

Suisse (Grisons): Parsennfurka près de Klosters, 2300–2400 m, 24.7.1954, v. Soest (h 33689).
Ensuite Davos, Laret, 1700 m, 1954 v. Soest (h); Valais: Champese, 1470 m, 1961, v. Soest (h).

Cette espèce n'est pas très rapprochée de *T. fasciatum* Dahlstedt; les feuilles sont plus étroites, les stigmates purement jaunes, etc.

Taraxacum iseranum van Soest **sp. nov.** (Fig. 7)

Planta robusta 15–40 cm alta.

Folia numerosa, erecta, sublute scentia, araneosa, (10–)15–20 (–30) cm longa (petiolo subangusto vel subalato ± purpureo inclusa), utrinque 4–6 loba; lobi laterales anguste triangulares vel falcati, acuminati, dorso saepe magis convexo interdum dentati vel fissi, summo retroversi vel patentes vel erecto-patentes, in interlobiis 4–5 mm latis dentatis ± abeuntibus; lobus terminalis sagittatus, lobuli basis saepe erecto-patentes, apice elongati acuti.

Scapi foliis aequilongi, inferne rubroviolacei, sub involucro dense araneosi.

Involucrum 15 mm longum 20 mm latum subobscure viride. Squamae exteriores reflexo-patentes late lanceolatae 9 mm longae, ad 4 mm latae, purpurascentes immarginatae apice purpureae.

Calathium ad 3 cm diametro. Ligulae obscure luteae, marginales planae, extus stria atro-violacea notatae. Antherae polliniferae; stylus et stigmata livescentia. Floret vere.

Achenium stramineum 4 mm longum (pyramide inclusa) ad 0,8 mm latum, superne grosse suberecto-spinulosum ceterum rugosum—basi laeve, in pyramiden brevam, 3 mm longam conicam abrupte abiens. Rostrum 6–7 mm longum, pappus albus 7 mm longus.

E sectione Vulgariorum Dahlstedt.

France (Isère): Massif de Belledonne, au dessus du Lac Luitel, fossé de la route avec Saxifraga rotundifolia L., 1300 m environ, 18.5.1932; L. Kofler (h. R. de Litardière, h. v. Soest).

Ensuite:

France (Savoie): Val d'Isère, pentes argilleuses dans le bois du Rogoney, 1900 m environ; 15.7.1958, B. de Retz 43718 bis.

Taraxacum lucidepedatum van Soest **sp. nov.** (Fig. 8)

Planta 5–8 cm alta, sat robusta, basi lucide alba vel rosea.

Folia numerosa subdecumbentia, canescenti-viridia glabra, 4–12 cm longa, lobata, petiolis basi late alatis nervoque mediano pallido vel roseolo-colorato; lobi laterales 1 (–2) cm longa, patentes, triangulares vel subfalcati, acuti, dorso minute dentati vel denticulati vel integres, margine inferiore rarissime denticulati; interlobiis ca. 1 cm longis, 1–2 mm latis, raro denticulatis; lobus terminalis integer, deltoideus vel subhastatus vel sagittatus, 5–15 mm longus, lobuli basis acuti, lobulo apicali interdum elongato, acuto.

Scapi paulo araneosi, foliis breviori.

Involucrum obscure viride, crassiusculum, ad 13 mm longum, ad 13 mm latum. Squamae exteriores patentes vel apice recurvatae, ovatae vel ovato-lanceolatae, ca. 7 mm longae, 2–4 mm latae, inconspicue albo-marginatae, omnes laeves.

Calathium planum radians, ad 3 cm diametro, luteum. Ligulae marginales planae, extus stria fusco-violacea notatae. Antherae vacuae; stylus et stigmata subnigra. Floret vere.

Achenium stramineum, 3 mm longum (pyramide exclusa), superne spinulosum, ceterum rugosum—basi laeve, in pyramiden conicam

0,2 mm longam subsensim abiens; rostrum 8–9 mm longum; pappus albus, 5 mm longus.

E sectione Vulgariorum Dahlstedt.

PORTUGAL (Estramadoura): Lisbonne, dans la pelouse du Jardin Campo Grande, 80 m altitude, 5.3.1949, Bento Rainha 1719, 1721 (h. LISE, h. v. Soest); j'ai signalé ces plantes à tort sous le nom de *T. acutangulum* Marklund dans: Agronomia Lusit. XIII (1951).

Ensuite:

ANGLETERRE (Surrey): Coulsdon, Bradmore Green, 25.4.1953, A. H. G. Alston 11897 (BM, h. v. Soest).

Taraxacum mediterraniforme van Soest **sp. nov.** (Fig. 9)

Planta gracilis, ca. 10 cm alta, glabra.

Folia numerosa, paulo canescentia, petiolis angustis ± roseo-violaceis. Folia interiora lingulata, lobata; lobi laterales (utrinque ca. 4–5) subfalcati retroversi, 1,5–2 cm longi, acuti vel subobtusi, subintegres; interlobiis angustis ad 3 mm longis; lobus terminalis subhastatus vel subdeltoideus, integer, lobuli basis retroversi, acuti vel subobtusi, lobulo apicali subobtuso vel obtuso.

Scapi foliis subaequilongi, subpallidi.

Involucrum sat parvum, ad 1,5 cm longum, 1 cm latum, viride. Squamae exteriores parvae ± adpressae, ovatae, ad 6 mm longae, 2,5 mm latae, conspicue anguste marginatae, obtusae, laeves; squamae interiores angustae, inferne sublate membranaceo-marginatae.

Calathium paulo radians, ad 2 cm diametro, luteum. Ligulae marginales extus stria obscure cano-purpurea ornatae. Antherae polliniferae; stylus et stigmata subobscura. Floret vere.

Achenium stramineum, 4 mm longum (pyramide inclusa), superne spinulosum, ceterum rugosum—basi laeve, in pyramiden conico-cylindricam 0,7 mm longam laevam subabrupte abiens; rostrum 6 mm, pappus niveus 5 mm longus.

FRANCE (Hérault): St. Guilhem-le-Désert, vallée du Verdus, 400 m, 21.5.1957, S. J. van Ooststroom 20463 & Th. J. Reichgelt (L).

Ensuite:

France (Var): Fréjus, Forum Julii, 1950 v. Soest (h); entre Fréjus et St. Aygulf, 1950 v. Soest (h); Castella d'Agay, 1950, v. Soest (h).

France (Gard): le Cailer, prairie du type Arrhenatherion, aspect de Narcissus tazetta L., 1959, M. Donker et A. Stevelink (SIGMA).

T. mediterraniforme est très rapprochée de *T. mediterraneum* v. Soest; les feuilles sont plus grisâtres, les capitules plus petits, les folioles involucrales extérieures plus petites et appliquées sur l'involucre, les akènes plus épineux; la hampe est glabre.

Le capitule est assez semblable à celui de *T. ruborum* v. Soest, mais les plantes sont de plus petite taille, les feuilles plus grisâtres, les pétioles moins pourpres, les akènes plus spinuleux; ensuite, la forme des feuilles est nettement différente.

Ces trois espèces appartiennent à un sousgroupe des *Vulgaria*, signalé dans la région méditerranéenne occidentale; en Portugal il est représenté par quelques espèces, tels que *T. algarbiense* v. Soest, *T. lusitanicum* van Soest et *T. triforme* v. Soest. Malheureusement, il

me n'est pas encore bien possible de délimiter ce sousgroupe en termes morphologiques suffisamment clairs.

Taraxacum ruborum van Soest **sp. nov.** (Fig. 10)

Planta gracilis 15–25 cm alta, subglabra.

Folia gramineo-viridia vel lutescentia, ad 22 cm longa, petiolo angusto vinoso-colorato inclusa. Folia exteriora subintegra—dentata, anguste obovata vel sublobata nervo saepe purpureo. Folia interiora lobata; lobi laterales (utrinque ca. 3) positi saepe asymmetrici ergo interlobiis brevis vel sublongis, raro dentatis, saepe pro parte corruptis; lobi laterales triangulares ad 3 cm longi, integres vel raro dorso saepe convexo 1 dente muniti, subobtusi interdum paulo acuminati; lobus terminalis elongato-hastatus 2,5–7 cm longis, ad 2,5 cm latis, integer subacutus, lobuli basis ad 2 cm longi subobtusi vel subacuti.

Scapi foliis aequilongi, purpurei glabri.

Involucrum basi rotundatum siccis atroviride. Squamae exteriores adpressae, ovatae breves (ad 6 mm longae), 3 mm latae, conspicue marginatae ciliolatae, apice purpureae obtusae; squamae interiores ad 12 mm longae, omnes laeves.

Calathium mediocre, paulo radians, ad 2 cm diametro. Ligulae saturate luteae, marginales planae, extus stria obscure cano-violacea notatae. Antherae polliniferae; stylus et stigmata obscura, siccis nigra. Floret vere.

Achenium stramineum ad 4 mm longum (pyramide inclusa) superne breve spinulosum ceterum sublaeve—laeve, in pyramiden subcylindricam 0,7 mm longam laevam abrupte abiens. Rostrum ca. 5 mm longum, pappus niveus, 5 mm longus.

France (Gard): le Cailer, prairie de type *Arrhenatherion* à aspect de *Trifolium pratense* L., 10.4.1959, M. Donker & A. Stevelink (h. v. Soest).

Ensuite:

Le Cailer, accompagné de *Carex divisa* Huds. ou de *Narcissus poeticus* L. et *Cirsium tuberosum* (L.) All. (h. VAD); de même dans une prairie légèrement saline, en compagnie de *Carex divisa* Huds. et *Leucojum aestivum* L. (h. v. S.), tous, 1959, M. Donker & A. Stevelink; Fréjus (Var), prairie marécageuse, non loin de la mer, 1950, v. Soest (h); Fontfroide (Hérault), 1840 .... sous le nom de *T. palustre* (BR).

SECT. ALPINA HAGLUND

Taraxacum authionense van Soest **sp. nov.** (feuille: Fig. 1)

Planta ca. 10 cm alta.

Folia luteo-viridia, petiolis subalatis nervoque mediano praesertim parte inferiore purpureo-colorato; folia exteriora subintegra, inferiora lobata; lobi laterales (utrinque ad 4) triangulares, ad 1 cm longi, integres, acuminati, $\pm$ retroversi; lobus terminalis triangularis, lobuli basis acuti, lobulo apicali subacuto vel subobtuso.

Scapi araneosi.

Involucrum ca. 10 mm longum, 10 mm latum, obscure viride. Squamae exteriores subadpressae, apice recurvatae, lanceolatae,

4 mm longae, 2 mm latae, anguste marginatae, apice purpureae, laeves. Squamae interiores lineares, membranaceo-marginatae, laeves.

Calathium planum, radians ad 3 cm diametro, luteum. Ligulae

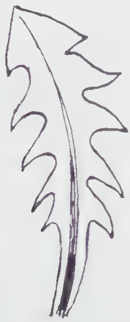

Fig. 1.
Taraxacum authionense v. S.; feuille.

marginales planae, extus stria cano-violacea notatae. Antherae ± vacuae, stylus luteus, stimata virescentia. Floret aestate.

Achenium ca. 3,5 mm longum (pyramide exclusa), ochraceum, superne spinulosum, ceterum rugosum, in pyramiden conicam 0,4 mm longam abrupte abiens; rostrum 5 mm longum; pappus albus, 4,5 mm longus.

E sectione Alpinorum Haglund.

FRANCE (Alpes Maritimes): massif de l'Authion, à environ 1 km de Cabanes Vieilles, 1875 m, 20.6.1958, J. Lambinon 58/M/1661; le no. 1662 contient des exemplaires juveniles à feuilles seulement grossièrement dentées.

Taraxacum pyropum van Soest **sp. nov.** (Fig. 11)

Planta parva, 4–7 cm alta, glabrescens.

Folia sublutescentia vel laete gramineo-viridia, ad 7 cm longa petiolo pallido subalato inclusa; exteriora anguste obovata integra vel subdentata, interiora lobata; lobi laterales pauci, deltoidei vel triangulares, ad 8 mm longi, subobtusi, integres; lobus terminalis brevus subdeltoideus, obtusus, integer.

Scapi 1–2, cuprei.

Involucrum parvum ad 12 mm longum, ad 12 mm latum, obscure viride, siccis subnigrum. Squamae exteriores subadpressae, ovatae, acuminatae, apice purpureo-marginatae, ad 5 mm longae; initeriores late membranaceo-marginatae, apice obscure purpureae.

Calathium subradians ad 2 cm diametro, croceum. Ligulae marginales angustae, saepe subclausae, extus stria purpurea (interdum atro-purpurea) ornatae. Antherae vacuae; stylus et stigmata lutea. Floret aestate.

Achenium maturum ignotum, ca. 4 mm longum, stramineum (?), superne paulo tuberculatum—breve spinulosum, in pyramiden brevam subsensim abiens; rostrum breve ca. 5 mm longum; pappus niveus, ca. 4 mm longum.

E sectione Alpinorum Haglund.

FRANCE (Isère): Huez, au lac Blanc, rive gauche, alt. 2640 m, 2.8.1959, R. Barbezat, exsiccat B. de Retz 3560 (sous le nom de *T. pyroporum*).

Ensuite:

Val Mesocco (San Bernardino), 2400 m, 1867 Brügger (K); Hautes-Pyrenées: Gèdre, 2150 m, 1959, de Retz 44667; Gavarnie, Bordère; Campvieil, Bordère (P).

T. pyrenaicum Reuter est une autre espèce selon la description originelle de l'auteur, mais Bonnier, Fl. de France, a employé le nom de *T. pyrenaicum* avec une description différente, mentionnant la couleur jaune des styles et les bandes d'un pourpre clair au côté de derrière des ligules, deux caractères se trouvant aussi dans la description de *T. pyropum*.

SECTIO FONTANA VAN SOEST (?)

Taraxacum graiense van Soest **sp. nov.** (Fig. 12)

Planta 5–15 cm alta, paula araneosa, basi subincrassata.

Folia numerosa, ad 15 cm longa, ad 4 cm lata, luteo-viridia, paulo canescentia, lobata, petiolis alatis rufescenti-coloratus vel pallidis. Lobi laterales utrinque ca. 4, falcati vel triangulares vel lingulati, interdum constricti, subacuti, patentes vel paulo retroflexi vel apice recurvati, saepe integres vel dorso saepe convexo (interdum magne) dentati; interlobiis latis (5–15 mm); lobus terminalis brevus (ad 2 cm longus) sagittatus vel deltoideus, acutus vel subobtusus, lobuli basis elongati ad 1,5 cm longi, acuti vel subacuti, patentes vel subretroversi.

Scapi ad 5, floriferi foliis breviori (vel subaequilongi?).

Involucrum crassum, ca. 15 mm longum, ad 20 mm latum, obscure ± rufo-viride, paulo glaucescente. Squamae exteriores laxe patentes (vel apice recurvatae?), ovatae vel ovato-lanceolatae, ad 8 mm longae, ad 4 mm latae, inconspicue anguste vel immarginatae, summo elongato obtuso, omnes laeves.

Calathium 4 cm diametro, luteum. Ligulae marginales planae, extus stria cano- vel fusco-violacea notatae. Antherae polliniferae; stylus et stigmata subobscure fuscescentia. Floret aestate.

Achenium (maturum ignota) stramineum, ca. 4 mm longum, superne spinulosum, ceterum rugosum—basi laeve, in pyramiden conicam (subsensim?) abiens. Rostrum ca. 5 mm longum; pappus albus, 6 mm longus.

FRANCE (Savoie): Val d'Isère, pentes argileuses dans le bois du Rogoney, altitude 1900 m env., 15.7.1958, B. de Retz 43717; de même: h. v. Soest.

Ensuite:

SUISSE (Grisons): Silvretta, Vernelatal, 2100 m, 18.7.1954, v. Soest (h).

AUTRICHE (Carinthie): Tauerntal, Feldseekopf, 1900–2000 m, 1.8.1957, v. Soest (h).

Cette espèce est nettement différenciée de *T. rufocarpum* v. Soest par les akènes straminés, les styles foncés et l'abondance de pollen. Pourtant, en état dépourvu d'akènes, les plantes des deux espèces peuvent être confondues fort aisément: les feuilles sont très semblables, ainsi que les involucres. *T. rufocarpum* est publié par moi dans Acta Bot. Neerl. 8 (1959) p. 124, où je l'ai provisoirement fait rentrer dans la sect. *Rhodocarpa* v.S., à cause de ses akènes d'une couleur plus

ou moins orangeâtre. Sans doute *T. rufocarpum* et *T. graiense* sont très rapprochées; la question reste encore ouverte à quelle section ces deux espèces appartiennent: sect. *Fontana* v.S.?

Je crois avoir signalé *T. graiense* dans d'autres endroits alpines en Suisse et en Tirol-méridional, mais l'absence d'akènes m'empêche de les publier ici.

Observation. Au lieu du nom *graiense*, j'ai d'abord donné le nom *graianum* à cette espèce.

SECT. ERYTHROSPERMA DAHLSTEDT EM. LINDBERG F.

Taraxacum lambinoni van Soest **sp. nov.** (Fig. 13)

Planta gracilis, ca. 10 cm alta.

Folia numerosa, laete gramineo-viridia, subtus $\pm$ araneosa, petiolis angusti, roseis. Folia exteriora lobata, lobi laterales (utrinque ca. 4) ad 8 mm longi, subovati, obtusi, integres vel 1 dente muniti, $\pm$ retroversi; lobus terminalis rotundato-obtusus, lobuli basis breves obtusi. Folia interiora lobata, lobi laterales (utrinque ca. 5) ca. 1 cm longi, falcati vel anguste triangulares, dorso saepe valde dentati, acuti vel acutissimi; lobus terminalis saepe elongatus, subobtusus, lobuli basis subacuti.

Scapi purpurei, araneosi.

Involucrum pallide viride, 12 mm longum, 10 mm latum. Squamae exteriores laxe patentes, lanceolatae vel ovato-lanceolatae, ad 7 mm longae, ad 2 mm latae, sublate albido-marginatae, laeves; squamae interiores callosae.

Calathium luteum; ligulae marginales extus stria cano-purpurea notatae. Antherae polliniferae; stylus et stigmata fusco-virescentia. Floret junio.

Achenium subpallide rufum, 3,5 mm longum (pyramide exclusa), superne argute spinulosum, ceterum rugosum—basi laeve, in pyramiden conico-cylindricam 0,8 mm longam sensim abiens; rostrum 7 mm longum; pappus sordide albus, 5 mm longus.

E sectione Erythrospermorum Dahlstedt em. Lindberg f.

FRANCE (Alpes Maritimes): Massif de l'Authion, prairie subalpine, rase, à environ 1 km de Cabanes Vieilles, près de la borne française, 1875 m, 20.6.1958, J. Lambinon (58/M/1663, 1667, 1668); de même les numéros 1664 (h. v. Soest) et 1666.

Taraxacum pseudo-dunense van Soest **sp. nov.** (Fig. 14)

Planta gracilis, ca. 10–15 cm alta, basi fragmentis foliorum vetustorum incrassata, subglabra sed scapi pro parte paulo araneosi.

Folia laet gramineo-viridia, tenera, petiolis angustissimis nervoque mediano vinoso-colorato. Folia exteriora lobata; lobi laterales anguste triangulares; folia interiora lobata, lobi laterales (utrinque ca. 5) anguste falcati vel sublineares, acutissimi, erecto- vel reflexo- patentes, saepe longe dentati vel denticulati; interlobiis saepe ad 1 cm longis, angustis, valde longe dentatis et denticulatis vel lobulo angustissimo saepe $\pm$ producto; lobus terminalis parvus, lobuli basis falcati vel

lineares, lobulo apicali lineare (ad 1 cm longo), ad 1 mm lato, acuto.

Scapi foliis aequilongi, vinoso-colorati.

Involucrum ca. 12 mm longum, ca. 12 mm latum, crassiusculum, obscure viride. Squamae exteriores adpressae, ovatae, ad 6 mm longae, ad 3 mm latae, late albo-marginatae corniculatae; squamae interiores late lineares, callosae vel corniculatae.

Calathium planum radians ad 3 cm diametro, saturate luteum; ligulae marginales planae, extus stria atro-violacea notatae. Antherae polliniferae, styli et stigmata livescentia. Floret vere.

Achenium fulvum, 3 mm longum (pyramide exclusa), superne spinulosum ceterum laeve, in pyramiden cylindricam 1 mm longam subsensim abiens. Rostrum 8 mm longum; pappus niveus, 5 mm longus.

E sectione Erythrospermorum Dahlstedt em. Lindberg f.

France (Basses-Alpes): Gorges du Verdon, 17.5.1959, S. J. van Ooststroom 21310 & Th. J. Reichgelt (L).

Les feuilles de cette espèce ressemblent légèrement celles de *T. dunense* v. Soest, ou même celles de *T. pseudo-lacistophyllum* v. Soest. *T. pseudo-dunense*, *T. dunense* et *T. pseudo-lacistophyllum* sont nettement différenciées par les akènes respectivement d'une couleur fauve, rouge et straminée.

Taraxacum pseudo-proximum van Soest **sp. nov.** (Fig. 15)

Taraxacum proximum Dahlstedt valde simile, sed squamae exteriores ovato-lanceolatae, 3–4 mm latae, rufo-purpureae, patentes apice recurvatae, conspicue anguste marginatae, callosae. Calathium 4 cm diametro; ligulae sublaete luteae; stylus luteus, stigmata paulo virescentia; antherae polliniferae. Achenium dilute brunneum.

E sectione Erythrospermorum Dahlstedt em. Lindberg f.

Pays-Bas (Sud-Hollande): île de Voorne, Nieuwenhoorn, 7.5.1956, K. B. van Brakel, P. D. Groot & J. L. van Soest (36432, de même: 36433, 36445, 36446).

Taraxacum retzii van Soest **sp. nov.** (Fig. 16)

Planta sat robusta ca. 20 cm alta, glabrescens.

Folia erecta vel suberecta, laete gramineo-viridia nervo mediano saepe purpureo, petiolis angustis, 2–4 cm longis, purpureis. Folia interiora lobata (utrinque 6–7 loba), lobi laterales triangularesvel late falcati, ad 2,5 cm longi, patentes vel paulo retroversi, acuti, integres vel raro (margine inferiore) dentati vel denticulati; interlobiis ad 5 mm latis, valde et saepe longe dentatis; lobus terminalis sagittatus, saepe acuminatus, acutus vel subobtusus, lobuli basis apice acuti.

Scapi inferne purpurei, araneosi, sub involucro dense araneosi, foliis subaequilongi.

Involucrum ca. 17 mm longum, ca. 19 mm latum, crassiusculum, obscure viride. Squamae exteriores reflexo-patentes, ovato-lanceolatae ad 4 mm latae ad 10 mm longae, sublate viride-marginatae, apice purpureae $\pm$ callosae.

Calathium ad 2,5 cm diametro, obscure luteum. Ligulae marginales planae, extus stria atro-violacea ornatae. Antherae polliniferae, stylus et stigmata fusco-virescentia. Floret vere.

Achenium (maturum ignota) ca. 3,5 mm longum (pyramide exclusa) fulvum, superne dense spinulosum, in pyramiden conicocylindricam 0,7 mm longam abrupte abiens. Pappus albus, 6 mm longus.

E sectione Erythrospermorum Dahlstedt em. Lindberg f.

FRANCE (Eure): Forêt de Vernon, 22.4.1959, B. de Retz 44411, pro parte.

Dans le sousgroupe *Fulva* Christiansen, *T. retzii* ne peut être confondue avec aucune des autres espèces: *T. atrosquamatum* v. Soest (Corse), *T. fulvum* Raunkiaer (Europe boréale et occidentale), *T. glauciniforme* Dahlstedt ap. Druce (Grande Bretagne et Irlande), *T. intercedens* Marklund (Esthonie et Suède), *T. isthmicola* Lindberg f. (Europe boréale), *T. maroccanum* Lindberg f. (Maroc), *T. oxoniense* Dahlstedt ap. Druce (Europe occidentale), *T. pseudo-dunense* v. Soest (France), *T. squamulosum* v. Soest (Corse).

Elle a un peu l'aspect de *T. polyschistum* Dahlstedt à akènes rouges.

NOTICE

Dans une étude sur les Taraxacum des Alpes, Acta Bot. Neerl. **8**, p. 59 (1959), il faut ajouter sous *T. panalpinum* v.S.:

"Typus: Silvretta: Vernelatal, 2250 m, July 1954, v. Soest (h. 33827)."

Fig. 2. *Taraxacum gallicum* v. S.; typus.

Fig. 3. *Taraxacum turfosum* (Sz. Bip.) v. S.; typus.

Fig. 4. *Taraxacum balearicum* v. S.; typus.

Fig. 5. *Taraxacum congestolobum* v. S.; typus.

Fig. 6. *Taraxacum fasciatiforme* v. S.; typus.

Fig. 7. *Taraxacum iseranum* v. S.; typus.

Fig. 8. *Taraxacum lucidepedatum* v. S.; typus.

Fig. 9. *Taraxacum mediterraniforme* v. S.; typus.

Fig. 10. *Taraxacum ruborum* v. S.; typus.

Fig. 11. *Taraxacum pyropum* v. S.; typus.

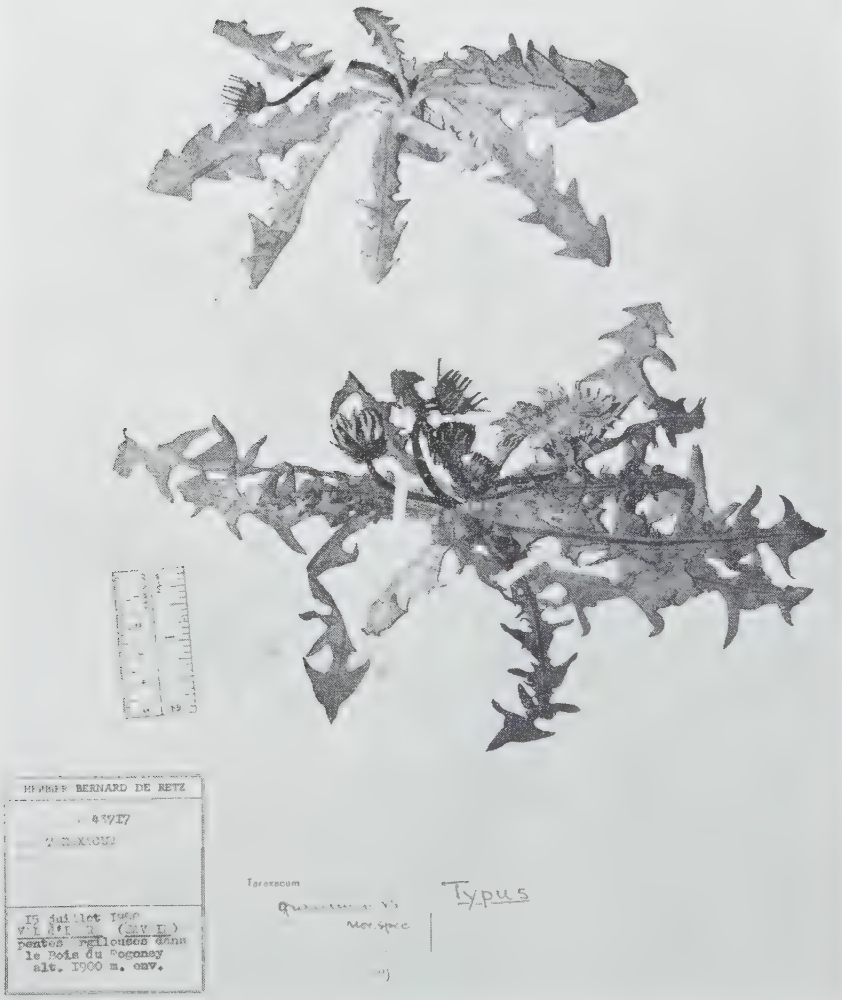

Fig. 12. *Taraxacum graiense* v. S.; typus.

Fig. 13. *Taraxacum lambinoni* v. S.; typus.

Fig. 14. *Taraxacum pseudo-duneuse* v. S.; typus.

Fig. 15. *Taraxacum pseudo-proximum* v. S.; typus.

Fig. 16. *Taraxacum retzii* v. S.; typus.

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ON THE IDENTITY OF THE GENERA MAPOURIA AUBL. AND GRUMILEA GAERTN. (RUBIACEAE, PSYCHOTRIEAE)

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In the course of my revision of the *Psychotrieae* of Madagascar, of which so far only a part has been published, I came across a comparatively large number of species which resembled each other in the presence of a ruminate endosperm and in the peculiarity that stipules are seen only at the tip of the shoots and, sometimes, at the base of the peduncles; on the vegetative part of the shoot they are rejected as soon as the pair of leaves which they enclose, begin to expand. As a ruminate endosperm and "deciduous" stipules are characteristic for a group of species to which now mostly the name *Grumilea* Gaertn. is applied, it seemed plausible to use this name also for these species of Madagascar.

The conclusion that the species of Madagascar meant in the preceding paragraph belong to *Grumilea* would be fully justified if we knew for certain that a ruminate endosperm and deciduous stipules were confined to this genus. This, however, is not so. My work on the *Psychotrieae* of tropical America has taught me that these characters are found also in the species of the genus *Mapouria* Aubl.

Whereas the use of the name *Grumilea* has always been restricted to species occurring in the tropical parts of Africa, Asia and the region further eastwards, the name *Mapouria*, which of late has been used for American species only, was applied by some of the older authors, like Hooker (in Benth. et Hook. f., Gen. Pl. **2**: 122. 1873) and K. Schumann (in Nat. Pflanzenfam. IV. **4**: 112. 1891) also to species occurring eastwards of the Atlantic.

Hooker, who knew that the *Mapouria* species are provided with deciduous stipules, but who was very badly informed with regard to the structure of their seeds and who undervalued the taxonomic importance of the differences that are found in the latter, included this genus in *Psychotria*, where he gave it the rank of a subgenus. In this subgenus he recognized two series, the "*Ebracteatae*" and the "*Bracteatae*"; to the *Ebracteatae* he referred besides a number of American species two entirely different Asiatic ones, viz. *Ps. tortilis* Bl., which is the type of the genus *Streblosa* Khs. (cf. Bremekamp in Journ. Arnold Arbor. **28**: 145–185. 1947), and *Ps. sarmentosa* Bl., and in the *Bracteatae* he included "mostly" paleotropic species, but as not one of those which he mentioned by name, is provided with deciduous stipules, it is difficult to see why this series which, to make matters worse, forms a rather heterogeneous mixture, was included by him

in this subgenus. Moreover, in not one of them the seeds possess a ruminate endosperm of the type found in *Mapouria* and *Grumilea* (v. infra).

Schumann too included in *Mapouria* a number of species found outside America, but he was apparently unaware of the fact that the type species has deciduous stipules, and that stipules of this kind are found also in the other American representatives of this genus. Of the Asiatic species which according to him would belong to *Mapouria*, only two are mentioned by name, viz. *M. fulva* (Ham.) K. Sch., a species which was included by Hooker in his series *Bracteatae*, and *M. connata* (Wall.) K. Sch. These species were apparently transferred from *Psychotria* to *Mapouria* because their seeds show no intrusion on the commissural side of the seeds, but although this is, as will be shown below, a character of considerable taxonomic importance, it is in itself insufficient to justify the transfer.

In view of the fact that *Grumilea* and *Mapouria* agree with each other in the presence of a ruminate endosperm and of deciduous stipules, it seemed desirable to see whether they agree also in other respects, and if so, what position they occupy on account of these points of resemblance with regard to the other genera of the *Psychotrieae*, especially with regard to those to which they seem to come nearest. If it would appear that they show a nearer affinity to each other than to any other genus, then we would be confronted with the question "are the two genera sufficiently distinct to be kept apart, or is it better to unite them?"

Schumann gives in his monograph of the Rubiaceae (Nat. Pflanzenfam. IV. 4. 1891) a key to the genera of the *Psychotrieae* (p. 110) in which *Mapouria* is contrasted with a group of genera in which *Grumilea* is included; in the latter the commissural side of the seed is said to be provided with a narrow fissure, whereas in *Mapouria* it is said to be entirely flat. This difference, which Müller-Argau had used already in his treatment of the *Psychotrieae* in the "Flora Brasiliensis" (VI. **5**: 383, 1881) in order to distinguish *Mapouria* from *Psychotria* and *Rudgea*, is doubtless of great taxonomic importance, as in all those genera of the *Psychotrieae* which may be regarded as well-defined, the seeds prove to be always of the same type, i.e. either in all species without an intrusion on the commissural side or in all of them with such an intrusion. Unfortunately Schumann made a serious mistake by including *Grumilea* in the group of genera in which the intrusion on the commissural side of the seed is always present. In reality this intrusion is in the seeds of *Grumilea* always absent, and in this important character there is therefore complete agreement between this genus and *Mapouria*.

The absence of an intrusion on the commissural side of the seed brings the genera *Mapouria* and *Grumilea* much closer together than they would have been if they had differed in this respect, and it accentuates more sharply the difference between them and the plants which were included by Schumann in the genus *Psychotria* and by Hooker, who, as we have seen, had put *Mapouria* as a subgenus in *Psychotria*, in the other subgenera of the latter.

To the three characters in which, as we now know, the genera *Mapouria* and *Grumilea* agree, another one may be added, viz. the heterostylism of the flowers.

In order to estimate the taxonomic value of these four characters, the deciduous stipules, the heterostylism of the flowers, the absence of an intrusion on the commissural side of the seed, and the presence of a ruminate endosperm, we will have to study the distribution of these characters among the related genera.

Deciduous stipules, i.e. stipules that are thrown off at the moment the next pair of leaves begin to expand, prove to be very rare indeed. Except in *Mapouria* and *Grumilea* they are known only from *Naletonia* Brem., a monotypic genus confined to Guiana and occupying a rather isolated position. It differs from *Mapouria* and *Grumilea* in the presence of a very deep intrusion at the commissural side of the seed and in the non-ruminate endosperm. To the diagnostic features of this genus belong the comparatively large bracts which are shifted from the base of the branchlet to the place where the next pair of branchlets is produced, and the imbricate aestivation of the corolla lobes. An imbricate aestivation of the corolla lobes is very rare in the *Psychotrieae*; in fact, it seems to be confined to this genus and *Notopleura* Brem. (v. infra). The deciduous stipules of *Mapouria*, *Grumilea* and *Naletonia* should not be confused with such stipules as are found e.g. in the genus *Chasallia* Comm. ex Juss. Stipules of that kind possess a marcescent upper part which is usually shed rather early, though not at the moment at which the next pair of leaves begin to expand; the non-marcescent basal part persists much longer.

The heterostylism, which in *Mapouria* as well as in *Grumilea* seems to be a general feature, is found also in the majority of the species which so far have been left in *Psychotria*. It proved to be present, for instance, in all the American species of this group which I could investigate. It is, moreover, a general feature in *Palicourea* Aubl., *Naletonia*, *Gamotopea* Brem., *Cephaëlis* Sw. and, probably, in *Notopleura*. *Naletonia* and *Notopleura* agree with *Mapouria* and *Grumilea* in one other taxonomically important character, *Naletonia*, as we have mentioned already, in the presence of deciduous stipules, *Notopleura* in the absence of an intrusion at the commissural side of the seed; in the other genera not a single of the taxonomically important characters of *Mapouria* and *Grumilea* is met with. In *Chasallia* heterostylous as well as isostylous species are found. In *Carinta* W. F. Wight (syn. *Geophila* Don), *Ronabea* Aubl., *Chytropsia* Brem. and *Nonatelia* Aubl. the flowers are always isostylous. As *Carinta*, *Ronabea* and *Chytropsia* agree with *Mapouria* and *Grumilea* in the absence of an intrusion on the commissural side of the seed, the absence of heterostylism in these genera is worth noting, because it accentuates the distance which separates these three genera from *Mapouria* and *Grumilea*, the more important differences being found in the persistent stipules and in the non-ruminate endosperm. On the whole, however, the presence or absence of heterostylism gives us but little information with regard to the degree of affinity between the various genera, and

this feature is in this respect doubtless of less importance than the three other diagnostic characters of the couple formed by *Mapouria* and *Grumilea*.

Seeds without an intrusion at the commissural side are found in some other genera too, viz. in *Ronabea*, *Notopleura*, *Carinta*, *Chytropsia* and *Gamotopea*, and further in a number of African and Asiatic species which so far have been left in *Psychotria*, although Schumann had referred at one time some of them to *Mapouria* (v. supra). However, when the genus *Psychotria* is subjected to a more thorough analysis than so far has been customary, these African and Asiatic species will doubtless be referred to one or more new genera. *Ronabea*, *Notopleura*, *Chytropsia* and *Gamotopea* are exclusively American genera, but *Carinta* is represented in Africa as well as in America, in Africa especially in the western part (it is found also in other tropical regions, but outside America and Africa as an introduced weed only). In none of these genera either deciduous stipules or a ruminate endosperm are met with (note, however, the restriction made further on with regard to *Gamotopea*). *Ronabea* differs moreover from *Mapouria* and *Grumilea* by the shape of its stipules, which are narrow and pointed, its few-flowered axillary inflorescences, its isostylous flowers, and the rather thick wall of the pyrene which on the convex side is provided with massive ribs; the commissural side of the pyrene, moreover, is not fully flat as in *Mapouria* and *Grumilea*, but slightly concave. In *Notopleura* the flowers are articulated with the top of the pedicel, the aestivation of the corolla lobes is not valvate, but imbricate, a character which seems to be confined to this genus and *Naletonia* (v. supra), and the pyrenes are dorsiventrally compressed. The species of *Carinta* are creeping herbs, a habit which is never met with in *Mapouria* and *Grumilea*, and the inflorescences are borne by axillary brachyblasts consisting of a single internode, the flowers are isostylous, the calyx lobes rather long, and the stamens included. It is noteworthy that among the Madagascar species to which I referred in the introductory paragraph of this paper, a small number proved to be provided with inflorescences borne by axillary brachyblasts, but whereas the brachyblasts of *Carinta* are always provided with well-developed and normally persistent leaves, they are in these species provided with rudimentary and short-living leaves. In *Chytropsia* the flowers are arranged in capitula which eventually may be arranged in an umbel, and they are, like those of *Ronabea* and *Carinta*, isostylous, but in this genus the stamens are not included but exserted. The species of *Gamotopea* are hirsute decumbent herbs with stipules which are divided in two linear or filiform lobes, with a calyx split to the base in narrowly triangular or filiform segments, and with pyrenes without any grooves or ribs. The structure of the endosperm is not yet known with certainty, as the seeds that could be investigated, were not fully ripe; however, as the proliferation of the spermoderm manifests itself, as a rule, already at a relatively early stage in the development of the seed, and as in this case no trace of such a proliferation could be detected, it does not look probable that the endosperm will become

ruminate. Among the species that so far have been left in *Psychotria* because they possess a non-ruminate endosperm, a small number are provided with seeds of which the commissural side is not provided with a longitudinal intrusion. This condition is found in the Asiatic species which Schumann referred on insufficient grounds to *Mapouria* (v. supra), and in some African ones like *Ps. pauridiantha* Hiern and *Ps. oddoni* de Wild. These species deserve a closer study, but it is now already quite clear that they can not be left in *Psychotria*, even if the latter is accepted in the rather wide delimitation proposed by Schumann, as the presence of an intrusion at the commissural side of the seed is regarded by the latter as an indispensable condition.

According to some remarks found in the literature it would sometimes be difficult to decide whether the endosperm is to be regarded as ruminate or as non-ruminate. In this connection it is worth noting that Hooker (l.c. p. 124) rejected the genus *Grumilea* because he was of opinion that it differed from *Psychotria* in one point only, viz. in the ruminate endosperm. This pronouncement, however, should not be taken too literally. As he certainly did not apply this rigorous, though undoubtedly fully justified condition in every case, it seems plausible to assume that his real motive is to be sought in a lack of confidence in the taxonomic value of this character. It is not improbable that this lack of confidence was due to some critical remarks made a few years before by MIQUEL in a paper (in Ann. Mus. Bot. Lugd.-Bat. **4**: 204. 1869) in which he reduced *Grumilea*, which at an earlier date had been accepted by him (Fl. Ind. Bat. II: 295. 1856), to the genus *Psychotria*.

In dealing with *Psychotria* Miquel remarked in this later publication "Ab hoc genere *Grumilea* Gaertn. nullo certe characteri differt. Corollae tubus longior vel brevior cum nulla alia differentia conjunctim occurrens characterem differentialem praebere nequit. Baccae costatae in utroque genere inveniuntur. Albumen *Grumileae* perperam ruminatum dictum potius cum semine lobatum vel costato-sulcatum est, tela tenui quae spermodermidem efficit inter lobos intrante, ruminatum dici nequit, quamvis in sectione transversa tale videatur. Eius modi seminum fabricam in plurimis *Psychotriae* genuinis speciebus observavi."

The two remarks with which Miquel begins, viz. that there is no general difference in length between the corolla of *Grumilea* and that of *Psychotria*, and that in both genera the fruits may be costate as well as ecostate, are correct, but this can not be said of his remarks on the structure of the endosperm. There can not be the slightest doubt that the endosperm of *Grumilea* is in this respect fully comparable to that of the nutmeg, and that the term "ruminate" is therefore fully justified. Nor can it be doubted that there is in *Grumilea* no direct connection between the intrusions of the spermoderm and the grooves by which the ribs on the surface of the seed are separated from each other. These grooves are not always present, but in the species in which they are found, they are confined to the convex side of the seed, whereas the intrusions of the spermoderm are found also

on the flat side; in fact, they may even be confined to that side. However, whether they are found on both sides or on the flat side only, they always appear to form a more or less regular network. On the commissural side this network can easily be exposed to view by rubbing the seed on a sheet of fine sandpaper.

Miquel was probably led astray by the existence of another type of ruminate endosperm in species belonging to this circle of affinity, though not to *Grumilea*. In this type we find indeed a relation between the proliferation of the spermoderm and the grooves between the ribs on the convex side, but as the species in which this type of ruminate endosperm occurs, are never provided with deciduous stipules and as the commissural side of their seeds is always provided with a longitudinal intrusion, they can not be included in *Grumilea*.

Miquel may also have been influenced by the fact that in a transverse section of the *Grumilea* seed we see at the commissural side, as a rule, but three or four intrusions of the spermoderm; this may create the impression that these three or four intrusions represent three or four longitudinal fissures, but this impression is entirely wrong; as soon as a tangential section is made, the presence of a more or less regular network is revealed.

The network mentioned in the preceding paragraph is present in the seeds of *Mapouria* too, though the meshes are here, as a rule, somewhat wider and less regular than in *Grumilea*, but as there is in this respect in both genera a good deal of variability, this difference is not very sharp. In *Pyragra* Brem., a genus of the *Psychotrieae* which is confined to Madagascar (cf. Bremekamp in Candollea **16**: 148 et Fig. 21. 1958), we also find a ruminate endosperm, and here the meshes are as small and as regular as in *Grumilea*, but this genus can nevertheless not be regarded as a very near ally, as the seeds show a deep intrusion on the commissural side and as the stipules are persistent. In the endosperm of *Pagamea* Aubl., an American genus which agrees with the paleotropic genus *Gaertnera* Lam. in the almost completely superior ovary and in the form of the stipules which are united in a high amplexicaul sheath, but which differs from *Gaertnera* in the ruminate endosperm, the meshes are even wider and less regular than they are in *Mapouria*.

The last remark made by Miquel, viz. that he had seen a similar structure of the endosperm in a large number of *Psychotria* species, rests doubtless, as indicated above, on a confusion of two types of ruminate endosperm, of which one occurs in *Grumilea*, *Mapouria*, *Pyragra* and *Pagamea*, whereas the other is found in a number of species that on account of the persistent stipules and the presence of an intrusion on the commissural side of the seed can not be included in *Grumilea* or *Mapouria*, and which therefore are provisionally retained in *Psychotria*. This second type occurs also in some species of *Cremocarpon* Boiv. ex Baill., viz. in *Cr. lantzii* Brem. and in *Cr. trichanthum* (Baker) Brem. (cf. Bremekamp in Candollea **16**: 148, 169 and 173. 1958). In the first type the intrusions of the spermoderm are completely independent of the grooves on the convex side of the seed,

which in the seeds of the plants where this type is found, may even be absent, and the intrusions are moreover best developed at or may even be confined to the commissural side, whereas in the second type the intrusions are mainly or exclusively found at the bottom of the grooves between the ribs on the convex side. "In plurimis *Psychotriae* speciebus" apparently should be understood as "in a large number of *Psychotria* species", not as "in most of the *Psychotria* species", as *Psychotria* species with a ruminate endosperm form after all but a small percentage of the large number of species which so far have been left in this genus. In none of the American representatives of this group I have ever seen seeds with a ruminate endosperm, and although such an endosperm is not very rare among its paleotropic representatives, the species in which it occurs form nevertheless but a minority. As an example I may quote *Psychotria vogeliana* Bth., a species which was included by Hooker in the series *Bracteatae* of his subgenus *Mapouria*. In the seeds of this species the commissural side is provided with two contiguous grooves, a structure which returns in most of the paleotropic *Psychotria* species, and its stipules are persistent. In this connection it is perhaps worth while to draw attention to the fact that Schumann committed a mistake when he said that *Grumilea aurantiaca* Miq. is a "*Psychotria*"; the deciduous stipules, the absence of an intrusion on the commissural side of the seed, and the presence of a network of spermoderm intrusions extending over the whole surface of the seed prove that it is a true "*Grumilea*".

The preceding exposition shows that *Mapouria* and *Grumilea* agree with each other in four important points, of which three, viz. the absence of an intrusion at the commissural side of the seed, the presence of a ruminate endosperm of a kind which differs from that found in some *Psychotria* species, and the presence of a type of stipules which is called "deciduous", deserve special attention, because in other genera of the *Psychotrieae* at the most but one of these characters is met with (deciduous stipules in *Naletonia*, the absence of an intrusion on the commissural side of the seed in *Ronabea*, *Notopleura*, *Carinta*, *Chytropsia* and *Gamotopea* and in a number of species which erroneously have been left in *Psychotria*, the presence of a ruminate endosperm of the same kind as that found in *Mapouria* and *Grumilea*, in *Pyragra* and in *Pagamea*). It is therefore impossible to sink *Mapouria* and *Grumilea* either in the "rump" genus *Psychotria* or in any other genus of the *Psychotrieae*.

Now that this point has been settled, we may turn our attention to the question whether *Mapouria* and *Grumilea* show differences of sufficient importance to justify their retention as distinct genera. If it proves impossible to find such differences, the two genera will have to be merged in one.

The best way to solve this problem seems to be to take a detailed description of one of these genera, and to compare it with a similar one of the other genus. The most recent description of *Mapouria* in which sufficient details are to be found, seems to be that which I myself have given in "Pulle, Flora of Surinam" (IV: 223. 1934). This

description is more suitable than that given by Müller-Argau in the "Flora Brasiliensis", because Müller-Argau included in this genus, apart from some insufficiently known elements, also a quite incongruous one, viz. that for which Don had proposed the already occupied name *Geophila*, and which is now known as *Carinta*. Of *Grumilea* no up-to-date description is available, and for this reason I will have to use instead the data which I myself have collected by the analysis of representatives of this genus occurring in various parts of its area of distribution.

My description of *Mapouria* reads:

"Glabrous or subglabrous shrubs, usually drying with a reddish brown tinge. Leaves opposite. Stipules rather large, thrown off when the next leaves expand, interpetiolar and simple. Inflorescence terminal, corymbose or paniculate, rarely two or three times umbellate; bracts and bracteoles present, but small, connected by a rim. Flowers sessile or shortly pedicellate, 5-merous, heterostylous. Ovary bilocular, with a solitary ascending ovule in each cell. Calyx truncate or shortly lobed, with or without glands on the inside. Corolla hypocrateriform or infundibuliform; tube in the upper half villous; lobes in the bud valvate with the tip bent inwards. Stamens inserted midway or somewhat above the middle of the tube; in the short-styled flower slightly exserted, in the long-styled one included; anthers dorsifixed. Disk entire, globose, conical or cylindrical. Style glabrous, filiform, ending in two linear lobes. Fruit a globose drupe with two pyrenes. Pyrenes sulcate on the convex side, entirely smooth on the flat side." We may add to this that the pollen grains are oblate and 3- or, occasionally, 4-porous, that the wall of the pyrenes is thin and corneous, and that the endosperm is ruminate, the intrusions of the spermoderm forming a network that is equally spread over the whole surface.

The species of *Grumilea* that were investigated by me, proved to be glabrous or hairy shrubs or, occasionally, suffrutices or small trees, and they too often assumed a reddish brown colour in drying. In these species too the leaves were always opposite, but this is of little importance, as species with verticillate leaves are very rare in the *Psychotrieae*. The stipules were rather large, and appeared to be shed when the next pair of leaves began to expand, but in contrast with those found in *Mapouria* they were, as a rule, bidentate to bipartite, though in a small number of species which seem to be confined to Madagascar and which on account of their large fruits occupy a somewhat isolated position in the genus, they seemed to be simple; this, however, is not fully certain, as in these species only the stipules at the base of the inflorescence were seen, and as these stipules are, as a rule, larger and less deeply incised than those on the vegetative part of the shoot, it is not entirely excluded that in these species too the stipules on the latter will prove to be incised; at any rate in these species too the stipules proved to be provided with two distinct keels converging towards the top, a character that is found in all paleotropic species. In *Grumilea* too the inflorescences proved to be terminal,

although in a small group of species which seem to be confined to Madagascar, it looked at first sight as if they were axillary; however, on closer inspection it appeared that they are borne here by axillary brachyblasts consisting of a single internode and provided with a pair of rudimentary, early deciduous leaves. The inflorescences, moreover, were always corymbose or, more rarely, paniculate, and the bracts and bracteoles proved to be small and often, though not always, connected by a rim. Here too the flowers were sessile or shortly pedicellate, usually 5-merous, though in one of the species found in Madagascar they proved to be 4-merous, and in another one 6- or 7-merous, and here too they were always heterostylous. The ovary was everywhere bilocular, and, as in all *Psychotrieae*, each locule contained a single ascending ovule. The calyx was shortly lobed, but with regard to the presence of glands on the inside I have no definite information, as this character, to which Müller-Argau attached great importance, is in my opinion not very reliable; for this reason I paid no attention to it. In *Grumilea* too the corolla proved to be hypocrateriform or infundibuliform, but this is of little importance, as these shapes return in most *Psychotrieae*; the tube was in the upper half or, more precisely, between the points of insertion of the stamens and somewhat above the latter provided with tufts of hairs, and as in the great majority of the *Psychotrieae* the lobes were valvate in the bud, with the tip bent inwards. The stamens proved to be inserted, as in most other *Psychotrieae*, in the middle or slightly above the middle of the corolla tube, and here too the anthers proved to be dorsifixed, but this too is a situation found in most *Psychotrieae*. Here too the pollen grains proved to be oblate and generally 3-porous. Here too the disk was found to be semi-globose, conical or cylindrical. The style was here too glabrous, filiform and at the top divided into two narrow lobes. Here too the fruit proved to be a globose or subglobose drupe with two pyrenes; the pyrenes had a thin and corneous wall, and they were either smooth or sulcate on the convex side and flat on the commissural one, and the endosperm was ruminate with intrusions of the spermoderm spread in the form of a network either over the commissural side or over the whole surface.

Apart from the difference in the structure of the stipules of which it is not fully certain that it is a general one, no differences of any importance are revealed in these descriptions, and the conclusion that the two genera are to be united, seems therefore unavoidable.

The conclusion that *Mapouria* and *Grumilea* are to be united, confronts us with a very difficult nomenclatural problem. Which name is to be applied to the combined genus?

As *Mapouria* Aubl. dates from 1775, and *Grumilea* Gaertn. from 1788, the problem seems to offer no difficulty, but a closer inspection reveals that the situation is less simple. The trouble is not caused by a difficulty in the identification of the type species. It is true that the identity of *Grumilea nigra*, Gaertner's type, is not absolutely certain, but there can be no doubt that it is either conspecific with or at least very nearly related to *Gr. nudiflora* Thw. (syn. *Psychotria thwaitesii*

Hook. f.), and with regard to the identity of *Mapouria guianensis* Aubl. there appears to be no uncertainty at all. The real trouble is found in the difficulty to find an answer to the question whether the genus proposed by Aublet may be regarded as a new one.

When we compare Aublet's generic description of *Mapouria* with that which Linné had given a few years before of *Psychotria*, it strikes us that there is not a single point of difference.

LINNÉ's description of *Psychotria* (Syst. Nat. ed. 10: 929. 1759) reads:

"Psychotria. Cal. 5-dentatus coronans. Cor. rotata. Bacca globosa. Sem. 2, hemisphaerica, sulcata.

Asiatica A. Psychotria stipulis emarginatis. Brown. jam. t. 17. f. 2.

AUBLET's description of *Mapouria* (Pl. de la Guiane I: 175. 1775) gives the following particulars:

"**Cal.** Perianthemum monophyllum, turbinatum, quinque-dentatum.

Cor. monopetala; tubus brevis, disco suprà germen insertus; limbus quinquefidus, lobis acutis.

Stam. Filamenta quinque, longitudine corollae, tubo inserta. Antherae subrotundae, biloculares.

Pist. Germen subrotundum, calici adnatum. Stylus oblongus. Stigma bilamellatum."

Fruit and seed were unknown to Aublet, but in the description of his only species, viz. *M. guianensis*, it is noted that the stipules are deciduous. The idea that this character might be of value for the recognition of the genus, did not enter Aublet's mind, as the latter, like most of his contemporaries, accepted the Linnaean fiction that the generic characters are to be derived from the organs of reproduction.

Linné's description of *Psychotria* and Aublet's description of *Mapouria* are both very incomplete, and apply to quite a number of genera in the *Psychotrieae* and, in fact, also to several Rubiaceous genera that fall outside this tribe. From a taxonomic point of view they are therefore of no value. The first acceptable definition of *Mapouria* was given more than half a century after the genus was proposed, by A. RICHARD (Mém. sur la famille des Rubiacées: 93. 1831), and of *Psychotria* even to-day no satisfactory definition is available. That Linné himself had no very clear idea with regard to the delimitation of *Psychotria*, follows from the fact that he afterwards included in it such a totally different plant as that described in 1760 by Jacquin as *Psychotria herbacea*, which at present is known as *Carinta herbacea* (Jacq.) W. F. Wight.

From a purely nomenclatural point of view the considerations given above are of no importance. To the nomenclaturist of to-day the only points which matter are whether for each of the genera a type can be indicated, and if so, whether these types are sufficiently different to be regarded as representing distinct genera. Now, with regard to the identity of the type of the genus *Mapouria* there is, as stated above, no doubt at all, but in the case of the genus *Psychotria* the position is unfortunately by no means clear.

With regard to the identity of the type species of *Psychotria* we have to rely on indications that are to be found in the generic name, the specific epithet, the, very laconic, diagnosis and the synonymy, and these indications prove to be contradictory. The generic name is a modification of the name "*Psychotrophum*" proposed by Patrick Browne for a plant from Jamaica, and points therefore to a species of American origin. The specific epithet "*asiatica*", on the other hand, points to an asiatic species. This is doubtless of importance, because in this circle of affinity the area of distribution of the species is on the whole very small; in fact, apart from one or two anthropochorous weeds there is not a single representative of the *Psychotrieae* which, so far as we know at present, occurs in both hemispheres. The diagnosis "Psychotria stipulis emarginatis" seems to exclude the possibility that it might be the plant described by Browne which, nevertheless, is quoted as a synonym. The plant of Patrick Browne, for which Sprengel introduced the name *Psychotria brownii*, is almost certainly a *Mapouria*, and its stipules therefore must have been entire; so they are indeed in the species to which this name is applied in Fawcett and Rendle "Flora of Jamaica". The name *Psychotria asiatica* is therefore clearly a nomen confusum.

It might perhaps be thought that the description of *Ps. asiatica* given in the same year 1759 in Gabriel Elmgren's "Pugillus Jamaicensium Plantarum" (reprinted in Amoenitates Academicae **5**: 395) would give us some information, but this is not so. It reads "26. *Psychotria asiatica*. Folia opposita, petiolata, lanceolata, integerrima, nuda. Thyrsi brachiati, e dichotomia caulis, foliis breviores." It contains therefore not a single item which might help us in our attempt to identify the plant.

Summarizing we may say that it is impossible to make out what species Linné had before him when he described the genus *Psychotria*, but that it is hardly believable that it would have been the Jamaican plant described by Patrick Browne. It seems more probable that it actually was an Asiatic plant, and that he erroneously assumed that the latter was conspecific with the Jamaican one of Patrick Browne. The identity of the specimen in the Linnaean herbarium which bears the name *Psychotria asiatica*, seems to be of little importance, as it will hardly be possible to prove that this specimen was already in Linné's possession when he described the genus, or that it was at that moment the only specimen he possessed. For the moment, however it seems to be sufficient to draw attention to the fact that Linné's attention had been drawn by the structure of the stipules. This makes it very probable that it was a plant with persistent stipules, for in a plant with deciduous stipules, he would hardly have noticed them. If this conclusion is right, the plant can not have been a *Mapouria*, and its affinity with the Jamaican plant of Patrick Browne must have been very remote.

It is perhaps of some interest to note that Aublet accepted the genus *Psychotria* and mentioned three species from French Guiana, viz. *Ps. violacea* Aubl. (l.c. p. 145 and tab. 56), *Ps. herbacea* L and *Ps.*

asiatica L. The two first-mentioned species are creeping herbs and belong to *Carinta*, but what he meant with *Ps. asiatica* is unknown. He describes it as a shrub, and as he quotes "Browne Jam. 160. t. 17. f. 2", it must have been a plant resembling the species described by Patrick Browne, but as he says that he had found this plant also in Ile de France, i.e. in Mauritius, the resemblance need not have been very strong. The Guiana plant may have been a species of *Mapouria*, but in that case it would have been a near ally of *M. guianensis*, the species on which the genus *Mapouria* was founded, and then it would be even more difficult to understand why he did found it.

More interesting is what Richard (l.c. p. 91) had to say on the genus *Psychotria*, as this author seems to have been the first who subjected it to a critical examination. *Chasallia*, *Ronabea*, *Cephaëlis*, *Mapouria*, *Palicourea* and *Naletonia* were accepted by him as generically distinct, but *Geophila* (i.e. *Carinta*) which had been split off by Don, was returned to *Psychotria*, and he also reduced *Grumilea* to this genus. The inclusion of these two genera is to be regarded as a mistake, for they do not agree with the generic description. As mentioned above, the seeds are in these two genera never provided with a longitudinal intrusion at the commissural side, and according to Richard this intrusion must be regarded as a general feature of *Psychotria*. The generic description does not apply to *Psychotrophum* Browne either, which Richard quotes in his synonymy, at least if we assume that the plant of Patrick Browne is identical with *Psychotria brownii* Spreng. as it is understood to-day, for in that species too the intrusion at the commissural side of the seed is absent (v. supra). A rather serious mistake is that the stipules of *Psychotria* are said to be entire, whereas in the species which he left in this genus they are in reality always bidentate to bipartite.

If we accept *Psychotria* in the delimitation given to it by Richard, then the name *Mapouria* may be retained for the species described under this name by Aublet and for those species which agree with the latter in the deciduous stipules, the heterostylous flowers, the absence of an intrusion at the commissural side of the seed, and the presence of an endosperm with a network of intrusions from the spermoderm either extending over its whole surface or confined to the commissural side.

It is in this connection perhaps noteworthy that the points of difference between *Mapouria* and *Psychotria* on which Richard himself laid special emphasis, viz. the much shorter corolla of *Mapouria*, its exserted stamens, its shorter anthers and its bearded corolla throat, are all of them illusory; in fact, in none of these points a constant difference between these genera exists. That he regarded the stamens of *Mapouria* as exserted and those of *Psychotria* as included, shows that he did not examine a very large number of specimens, otherwise he would have noted that the flowers are everywhere heterostylous, and that the stamens are in the dolichostylous flowers always included and in the brachystylous ones always exserted. This mistake, however, is excusable, as heterostylism, although observed already by C. K.

SPRENGEL (Das entdeckte Geheimniss der Natur: 103, 1793) in *Hottonia palustris*, was in Richard's time generally overlooked; in fact, its importance was not recognized before 1877 when DARWIN published his "Different Forms of Flowers in Plants of the same Species".

SUMMARY

The generic name *Mapouria* Aubl. should be applied to those *Psychotrieae* in which the following set of characters is found: deciduous stipules, heterostylous flowers, seeds without a longitudinal intrusion on the commissural side and an endosperm in which the spermoderm penetrates in the form of a network which may be confined to the commissural side but which, as a rule, extends over the whole surface. This means that it should be used also for those species which up to now have been included in *Grumilea* Gaertn. It need not be given up in favour of *Psychotria*.

The name *Psychotria* may provisionally be retained in the conventional sense, with the proviso, however, that species with deciduous stipules or without a single or double longitudinal intrusion at the commissural side of the seed should be excluded. The endosperm may be ruminate, but the intrusions of the spermoderm should be confined to the bottom of the grooves on the convex side. The choice of a type species for this genus is better postponed until a decision has been reached on the question whether this group of species may be regarded as a natural one.

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AN INVESTIGATION OF THE PLANKTONIC POPULATION OF DIATOMS OF THE "ZANDKREEK" DURING THE PERIOD MARCH 1959 TO MARCH 1960

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Introduction

In the province of Zeeland the State Department for the Maintenance of Ways and Waterworks has planned, as part of the so-called "Three-Islands-Project", for Noord Beveland, Zuid Beveland and Walcheren—, two dikes in a channel called the Zandkreek, which runs between the three islands. At the west end of the channel, off Veere, there will be a big sea dike, and a smaller dike at the east end, where the Zandkreek discharges into the East Scheldt. On May 4th 1960 the east dike was completed, and the west dike has been completed except for a gap of 150 metres. In the spring of 1961 this gap will be closed and thereafter the tidal flow will no longer occur in the Zandkreek. In the future "Lake of Veere" the mean temperatures of the water will be higher in the summer and lower in the winter than in the adjoining North Sea and East Scheldt. It is expected that the concentration of salt will decrease only slowly because there will be no fresh water supply except rainfall. A few pumping engines, which pump water from the nearby poulders, deliver brackish water only and the "Walcheren Channel" is brackish as well.

It will be clear that the ecological conditions in the Zandkreek will be changed by both the factors just mentioned, salt and temperature, after the completion of the dikes, and this will have an influence on the flora and fauna present in this area.

Because these changes were expected an investigation was started just a year before the first sealing off to follow the changes in the flora. This investigation was at first restricted to the planktonic Diatoms and extended to other groups of plants and animals later. In the Diatoms a cycle of a year was investigated during the period March 1959 to March 1960.

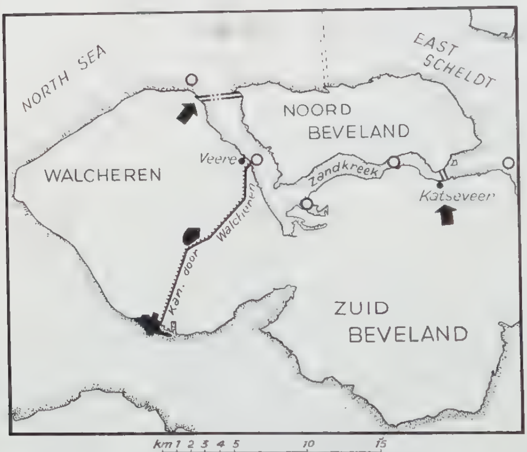
Methods

Five points were selected for monthly sampling, three lying within the area to be enclosed and two outside it, (see map). Measurements were made at these points of the temperature and the concentration of salt in the water. Samples were taken both at high tide and low tide, so that ten samples were taken each month. The samples were taken by filtering ten pails of water, equalling about 100 litres of

	1959 March 4th	April 3rd	May 1st	June 1st	June 30th	July 30th	August 27th	Sept. 28th	Nov. 26th	1960 Jan. 8th	Febr. 8th
Asterionella japonica Cl.	r	+	+	+	+	rr	r		rr	rr	rr
Bellerochea malleus Brightw.	rr	rr	rr	rr			r	r	rr	rr	r
Biddulphia aurita (Lyngb.) Bréb.	+	+	r	rr	rr			rr	r	r	c
B. granulata Rop.	r	rr	r	rr	rr	rr			rr	r	r
B. rhombus (E.) W.Sm.	r	r	rr	rr	rr	rr	rr	rr	rr	rr	rr
B. regia W.Sm.	+	+	r	+	+	r	r	r	r	r	+
B. sinensis Grev.	r	r	rr	+	+	r	c	cc	c	c	+
Cerataulina bergoni Per.	rr	r		r						rr	rr
Chaetoceros danicus Cl.	r	r	rr			rr	rr		rr		rr
C. decipiens Cl.	rr	rr									
C. radians Schütt.	+	r	rr	rr	rr	r	c				
Dytilum brightwelli (West) Grun.	+	r	r				r		r	r	rr
Coscinodiscus granii Gough	rr			rr	rr	rr	r	+	r	rr	
Eucampia zoodiacus E.	+	+	rr	cc	rr		r				
Guinardia flaccida (Castr.) Perag.	r	rr		rr	cc	+	rr	rr	rr	rr	rr
Porosira glacialis (Grun.) Jörg.	+	cc	rr	rr							
Rhizosolenia imbricata Shrubsolei (Cl.) Schr.	r	r	rr	+	rr	rr	+		r	r	r
R. habetata semispina (Hensen) Gran	r	r	r	r	rr	rr			r	r	rr
R. stolterfothi Perag.	rr	r		rr	r	rr	+		rr	rr	rr
Streptotheca thamesis Shrubs.	r	r	rr	rr					rr	+	+
Sceletonema costatum (Grev.) Cl.	+	+	+	rr	rr				rr	rr	rr
Thalassiosira nordenskiöldi Cl.	rr	r									
Chaetoceros debilis Cl.		r	rr								
C. curvisetus Cl.			rr				rr				
Thalassiosira decipiens (Grun.) Jörg.	rr	r									
Actinocyclus roperi Cl.				1ex						1 ex	
Chaetoceros densus Cl.				rr	r	+	r	rr		rr	rr
Bacteriastrum hyalinum Laud.					rr	+	r	rr	rr		
Chaetoceros lorenzianus Grun.					rr		r		rr	rr	
Coscinodiscus concinnus W.Sm.					rr	rr	rr	r	rr	rr	
Lauderia borealis Gran					r	r	r			r	
Leptocylindrus danicus Cl.					rr		r				
Chaetoceros affine Willei (Gran) Hust.						+	r				
C. dydimus E.						rr	rr		rr	rr	rr
C. costatus Pav.						c	rr				
C. compressus Laud.						+	r				
Coscinodiscus gigas var. ***praetexta*** (Janisch) Hust.							rr	r		rr	rr
Chaetoceros anastomosans (Gran) Hust.							+				

water, through a plankton-net, the width between the meshes being 70μ.

Map. 1. Map of the Netherlands with area investigated.



Map 2. Area investigated. ○ point of sampling. ↗ new dike.

Results

A survey of the planktonic Diatoms which occurred is now presented. The following symbols are used to represent the estimated abundance:

	approximate percentage of the planktonic Diatoms:
rr = very rare	1– 5
r = rare	5– 25
+ = common	25– 50
c = very common, not dominating in the sample	50– 75
cc = very common, dominating in the sample	75–100

The data in the following list give the mean value of the ten samples. In nearly all cases, all the samples from one sampling date were similar to each other.

In these results there are some points which merit particular attention. *Eucampia zoodiacus* E. is present in large quantities on June 1st and dominates all other species then, including *Guinardia flaccida* (Castr.) Perag. A month later this latter species shows a maximum and *Eucampia zoodiacus* E. can scarcely be found. Competition may be a cause of this quick succession of the maxima. At the sampling on July 30th three *Chaetoceros*-species appear at the same time and constitute the greater part of the plankton. These species are *Chaetoceros affine willei* (Gran) Hustedt, *C. compressus* Laud. en *C. costatus* Pav. The latter seems to have a more southern distribution.

A remarkable find is *Coscinodiscus gigas* var. *praetexta* (Janisch) Hustedt, which was present regularly in small quantities from the end of August onwards. It is a new species for the Netherlands. Hustedt (1930) mentions this species as occurring in the Mediterranean Sea. This suggests that the warm summer of 1959 was responsable for the very northerly occurrence of this species: the temperature of August 29th was 20,4° C. However this species was found in the samples of January 8th and February 8th when the temperatures were 6,4° C and 3,3° C respectively.

Fig. 1 represents a cell, × 400

Fig. 2 represents various drawings of a cell.

1. part of the disc.
2. areoles near the centre of the disc.
3. chromatophores.
4. pleura seen in face view.

In contradiction to what Hustedt writes, the valva is obviously concave.

Another species new for the Netherlands is *Porosira glacialis* (Grun.) Jörgensen, which was found in very large quantities (cc) on April 3rd. Hustedt (1930) mentions the species as "vorwiegend neritisch an den Küsten des nördlichen Eismeeres besonders im Winter auch an den südlicher gelegenen Küsten (Westküste Norwegens)".

Fig. 3 represents a part of the disc.

Fig. 4 represents a cell, magnification 1000 times.

In addition to the description of this author it was found that:

1. the areoles on the disc from the centre to the border are somewhat undulate and not in straight radial rows.

Fig. 1. Cell of Coscinodiscus gigas var. praetexta. (J.) Hust.

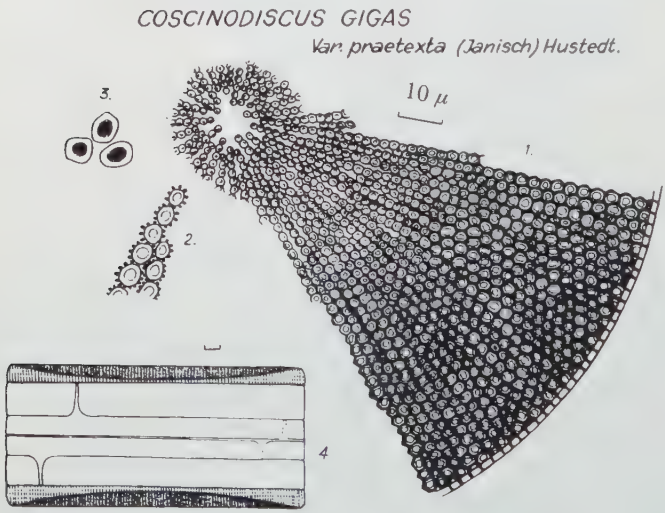


Fig. 2. Coscinodiscus gigas var praetexta. 1. part of the disc; 2. areoles near the centre of the disc; 3. chromatophores; 4. pleura seen in face view.

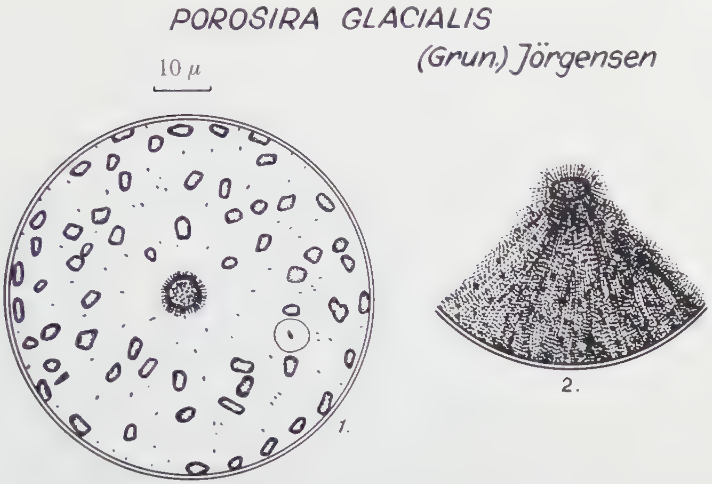


Fig. 3. Porosira glacialis (Grun.) Jörgensen. Part of the disc.

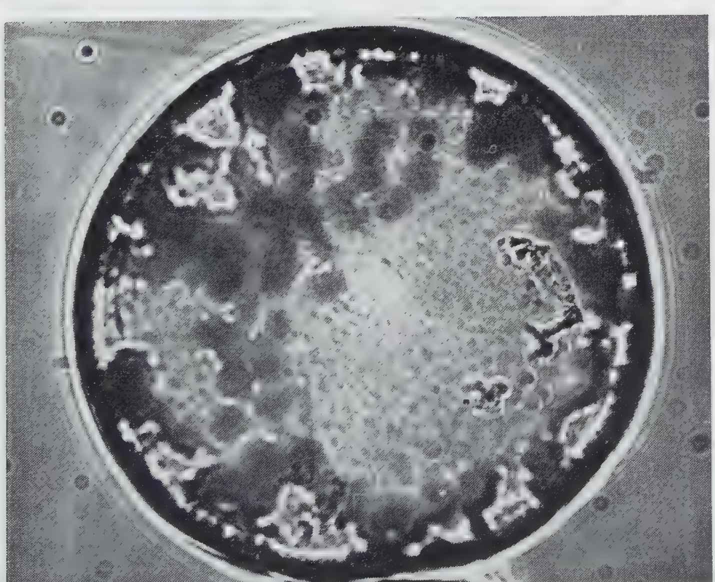


Fig. 4. Porosira glacialis (Grun.) Jörgensen. Cell.

2. near the centre of the disc the areoles have the form of a dash, pass gradually into points towards the edge and are arranged irregularly there.
3. in the centre an area is to be found in which there are a number of point-like areoles; the form is not constant.
4. no trace was found of jelly, connecting the cells.

When the Zandkreek is cut off at its eastern end in the spring of 1961, the question will be how the plankton will develop on each side of the dikes. After the sealing off a unique situation will arise for ecological hydrobiological investigation; the plankton of the Zandkreek will be compared with that of the North Sea on the one hand and that of the East Scheldt on the other.

ACKNOWLEDGEMENT

This investigation has been carried out at the Botanical Museum in Utrecht, Director Professor Doctor J. Lanjouw, with the kind help of Mr. A. van der Werff of the Geologische Dienst in Haarlem and with the co-operation of the Delta-Institute in Yerseke, set up by the Koninklijke Nederlandse Akademie van Wetenschappen, and with the Government Institute for Fishery Investigation Sublaboatory Wemeldinge (Zeeland).

SUMMARY

In a channel, which will be cut off soon, an investigation has been started in rihich the influence of the changing ecological factors will be studied. A ar's cycle of Diatoms, investigated in the period March 1959 to March 1960 elded some interesting results. In early June *Eucampia zoodiacus* E. showed a ≲aximum, whereas *Guinardia flaccida* (Castr.) Perag. showed its maximum in July, mhen *Eucampia zoodiacus* E. was in its turn rare.

Porosira glacialis (Grun.) Jörgensen, which comes from more Northern areas showed a maximum in early April.

Coscinodiscus gigas praetexta (Janisch) Hustedt appeared regularly from late August, (temp. 20,4° C), until February 1960 (temp. 3,3° C). Hustedt mentions this species as occurring in the Mediterranean Sea.

Some additions are made to the existing descriptions of the two last mentioned pecies.

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DAS FRÜHLINGSBILD DES DIPLOTAXIDETUM ERUCOIDIS BR.-BL. 1931

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(*eingegangen am 15. Mai 1961*)

Einleitung

Während eines Studienaufenthaltes an der Station Internationale de Géobotanique Méditerranéenne et Alpine in Montpellier, Direktor Professor J. Braun–Blanquet, wurden vom Verfasser in Süd-frankreich, (Languedoc), in den Weinbergen der Umgebung Montpelliers sowie im Departement Pyrenees Orientales 72 pflanzensoziologische Aufnahmen gemacht.

Nach J. Braun–Blanquet gehört die Vegetation der Weinberge des Languedoc zu der Assoziation Diplotaxidetum erucoidis (Br.-Bl. 1931). J. Braun–Blanquet hat hauptsächlich in den Jahren 1929–1938 in den Weinbergen des Languedoc 36, noch nicht publizierte, Aufnahmen gemacht; später, 1949–1952, kamen noch einige weitere hinzu. Es handelt sich dabei fast ausschliesslich um Herbst-Aufnahmen. In der ersten Periode:

2 Aufnahmen vom September
13 Aufnahmen vom Oktober
7 Aufnahmen vom November
5 Aufnahmen vom Dezember
2 Aufnahmen vom Januar
2 Aufnahmen vom April
1 Aufnahme vom Mai

In der zweiten Periode:

1 Aufnahme vom Mai
2 Aufnahmen vom Oktober
1 Aufnahme vom November

Meine Aufnahmen wurden alle im Frühjahr 1960 gemacht, um den Frühlingsaspekt dieser Assoziation kennen zu lernen.

In seiner ersten Übersicht der "Groupements végétaux du Bas-Languedoc" 1931, rechnet Braun–Blanquet die Assoziation von *Diplotaxis erucoidis* und *Amaranthus delilei*, (Verband *Diplotaxidion*), noch zu den *Secalinetalia*. Bei der Bearbeitung des Materials zum "Prodrome des groupements végétaux de la France Méditerranéenne" (1952), wurde das *Diplotaxidion* auf Grund neuer Aufnahmen und Erkenntnisse zur Ordnung *Chenopodietalia* gezogen so dass sich heute die folgende Einteilung ergibt:

Klasse	Ordnung	Verband	Assoziationen
Chenopodietea	*Chenopodietalia*	*Diplotaxidion*	*Diplotaxidetum erucoides*
			Eragrostideto-Chenopodietum
			Cynodonteto-Salsoletum

Ein erstes Optimum der Entwicklung der Assoziation von *Diplotaxis erucoidis* kann nach der Weinlese, im September bis November, beobachtet werden. Ein zweites liegt von Februar-April, nachdem die Annuellen sich entwickelt haben.

Methodik

Die Grösse der homogenen Aufnahmeflächen beträgt durchwegs 100 m²; die Form derselben ist immer quadratisch. Stets wurde in unmittelbarer Umgebung die Vegetation auf nicht im Quadrate vorkommende Arten untersucht und die neu gefundenen dazu notiert; sie sind in der Tabelle in Klammern () gesetzt; die gesammte Aufnahmefläche ist also grösser und beträgt etwa 300 bis 400 m². Die Aufnahmen wurden im Zentrum des Weinberges gemacht.

Bearbeitung der Weinberge, Düngung

Wo immer möglich wurde der Besitzer des Weinberges nach der Zeit der letzten Bearbeitung gefragt. Es stellte sich dabei heraus dass ein Weinberg im Allgemeinen drei bis vier Mal pro Jahr umgearbeitet wird. Dabei werden zwei Arten der Bearbeitung unterschieden:

a. eine Bearbeitung zwischen den Reihen der Weinstöcke,
b. eine totale Bearbeitung, das heisst eine zwischen den Weinstöcke selbst.

Bearbeitung b. findet im allgemeinen Januar-Februar, Bearbeitung a. nach der Weinlese statt. Eine Anzahl der Weinberge, die in Februar umgearbeitet wurden, zeigten Anfangs April keine Spuren einer Bearbeitung mehr, so das man sagen darf das die Vegetation sich innerhalb von etwa zwei Monaten völlig regenerieren kann. Einige Ausnahmen wurden jedoch beobachtet. Ein Weinberg, umgearbeitet in Dezember 1959 zeigte in April noch die Spuren dieser Bearbeitung; ein anderer, Anfang April umgearbeitet, zeigte in Mai schon wieder eine homogene Vegetation. In wie weit die Angaben der Weinbauern zuverlässig sind, ist nicht zu sagen; sie dürften aber wohl stimmen.

Die Bearbeitung geschieht in der Regel mit Traktoren. In Ausnahmefällen wurde auch die Verwendung von Pflug und Handhacke beobachtet, in letzterer Fall wo es sich um ganz kleinen Flächen handelte. In diesem Zusammenhang wäre es interessant zu untersuchen ob die Art der Bearbeitung einen Einfluss auf die Vegetation ausübt. Mit Ausnahme von 9 Aufnahmen wurden alle Quadrate nach etwa 10–16 Tagen wieder besucht. Eine Anzahl der Weinberge war inzwischen umgearbeitet worden. Dabei konnte festgestellt werden dass besonders *Convolvulus arvensis* L. und *Malva silvestris* L. eine Bearbeitung des Bodens gut ertragen und sich schnell regenerieren.

Düngung

Die Düngung besteht aus Stallmist, Kunstdünger oder verrotteten Abfällen jeder Art. Die Besitzer der Weinberge wurden immer nach dem Zeitpunkt und der Art der letzten Düngung gefragt, doch sind die Angaben besonders was die Art der Düngung anbelangt, nicht immer zuverlässig.

Kalkgehalt und pH des Bodens

Wie man aus der Tabelle ersehen kann, variiert der Kalkgehalt von 0–70 %. Jedoch ist es bei meinen Aufnahmen in der Umbegung Montpelliers kaum möglich einen Einfluss des Kalkgehaltes auf die Vegetation nachzuweisen. Eine Beziehung zwischen Kalkgehalt und pH ist nicht festzustellen; letztere schwankt zwischen 6,2 und 8,3.

Wenn wir die 36 Herbst- und 4 Frühlingsaufnahmen von Braun–Blanquet mit den 52 im Frühling 1960 gemachten Aufnahmen vergleichen, bekommen wir folgendes Bild.

Assoziations-Charakterarten (s. Tabelle 1)

36 Herbst, 64 Frühlingsaufnahmen J. Br.–Bl. 52 Frühlingsaufnahmen H. Stekhoven

	H	F	
Setaria verticillata (L.) P.B.	29	1	0
Diplotaxis erucoidis DC	25	2	25
Aristolochia clematitis L.	15	3	13
Solanum nigrum L. em. Mill.	16	0	1
Amaranthus silvestris Desf.	16	0	0
Veronica persica G.Mel.	7	2	10
Eragrostis Barrelieri Daveau	5	0	0
Xanthium orientale L.	5	0	6
Sorghum halepensis Pers.	2	0	0

Es stellt sich also heraus, dass nur bei den kursivirten Pflanzenarten die Stetigkeitswerte einigermassen übereinstimmen. Die anderen Charakterarten, wie sie von Braun-Blanquet angegeben sind, waren nicht zu finden.

Es ist erwähnenswert, dass *Xanthium orientale* L. als Keimpflanze zum ersten Mal am 16. April gefunden wurde. Bei der Kontrolle stellte sich heraus dass um den 20. April in 4 Aufnahmen Keimpflanzen gefunden wurden. Auch *Aristolochia clematitis* L. erscheint in dieser Zeit an der Erdoberfläche. Bei der Kontrolle konnte in 8 Fällen diese Pflanze nachgewiesen werden.

Zusammenfassend kann man sagen dass von den 9 Charakterarten des *Diplotaxidetum erucoidis*, wie diese von Braun–Blanquet angegeben wurden, 5 im Frühjahrsaspekt der Assoziation gar nicht gefunden wurden. Der Vergleich zwischen den Aufnahmen von Braun–Blanquet und meinen Frühjahrsaufnahmen zeigt nicht nur bei den Assoziationscharakterarten einen grossen Unterschied, sondern auch bei den Verbands- und Ordnungscharakterarten. Das zeigt die folgende Tabelle:

36 Herbst- 4 Frühlingsaufnahmen J.Br.–Bl. 52 Aufnahmen H. Stekhoven (s. Tabelle 1)

	H	F	
Solanum alatum Moench	23	0	0
Heliotropum europeum L.	18	0	0
Amaranthus albus L.	17	0	0
Fumaria micrantha Lag.	2	0	5
Eragrostis major Host.	2	0	0

Charakterarten der Ordnung

Amarantus retroflexus L. var. Del.	32	0	0	
Setaria viridis P.B.	22	0	0,7	Tote ex.
Rumex pulcher L.	17	1	26	
Portulaca oleracea L.	16	0	0	
Digitaria sanguinale L. (Scop.)	10	0	0	
Avena sterilis L.	4	2	0	
Diplotaxis muralis (L.) DC.	4	1	0	
Euphorbia peplus L.	1	0	0	
Xanthium spinosum L.	3	0	0	
Centaurea calcitrapa L.	2	0	0	
Sambucus ebulus L.	1	0	0	

Von den 5 Verbands-Charakterarten wurden 4 und von den 11 Charakterarten der Ordnung wurden 10 nicht gefunden.

Das Frühlingsbild ist also völlig verschieden vom Herbstbild. Es ist schade dass die Aufnahmeorte nicht im Herbst wiederbesucht werden konnten, um einen Vergleich zwischen Frühlings- und Herbstaufnahmen in einunddemselben Jahr durchzuführen zu können und um die neuen Ergebnisse mit denen von Braun–Blanquet zu vergleichen, die sich auf Sommer- und Herbstaufnahmen beziehen, während sich die Assoziationen in optimaler Entwicklung befindet.

Die Aufnahmen in Banyuls (s. Tabelle 2)

Der Badeort Banyuls liegt in den östlichen Pyrenaeën etwa 20 km von der Spanischen Grenze entfernt. In der Umgebung diesen Ortes wurden in den Weinbergen Aufnahmen gemacht um diese mit diejenigen von Montpellier zu vergleichen. Die klimatologischen und edaphischen Factoren sind von denen des Languedoc deutlich verschieden, was auch in der Vegetation der Weinberge zum Ausdruch kommt. Erstens ist hier, in diesem südlicher gelegenen Gebiet Frankreichs, die Jahrestemperatur höher und zweitens ist der Boden völlig kalkfrei.

In der Umgebung von Banyuls werden die Weinberge im allgemeinen zweimal pro Jahr umgearbeitet. Die Unkräutern werden bei diesen Bearbeitungen, die im Juli-August, vor der Weinlese, und im Januar-April stattfinden, meistens aus den Weinbergen entfernt.

In den Aufnahmen von Banyuls kommen viel vor: *Rumex bucephalophorus* L. und *Chrysanthemum segetum* L. Beide Arten dominieren im Frühjahr in den Weinbergen von Banyuls: aus der Ferne sahen nicht umgearbeitete Felder braun oder gelb aus. Die Gesellschaft dieser Weinberge ist der Assoziation von *Chenopodium botrys* und *Eragrostis major* (Br.-Bl. 1936) zuzurechnen.

Braun–Blanquet beschreibt sie für Weinberge auf kalklosen und mehr oder wenigen sandigen Boden im Bassin de L'Orb und gleichfalls aus der Gegend von Banyuls. Es ist interessant dass Braun–Blanquet ebenfalls im "Prodrome des Groupements végétaux (Fasc. 3) 1936, pg 26, eine Aufnahme aus Blanès (Catalogne) aufführt in der *Rumex bucephalophorus* L. und *Chrysanthemum segetum* L. reichlich vorkommen.

Uber die soziologische Stellung der Aufnahmen lässt sich nichts

TABELLE 1 Diplotaxidetum erucoidis Br.-Bl. 1931 52 Aufnahmen aus der Umgebung von Montpellier

DIPLOTAXIDETUM ERUCOIDIS Br.—Bl. 1931

Front. = Frontignan · G.B.B. = Garten Br.Bl. · St.B. = St. Bauzille-de-Putois · Montp. = Montpellier · Montf. = Montferrier · Lam. = Lamour · Masd.L. = Mas-de-Londres · Vend. = Vendargues

Stelle, Ortslage	St.B.	St.B.	St.B	St.B.	St.B.	St.B.	St.B.	Lam	Montp.	Montp.	Montp.	Montp.	Montp.	Montp.	Montf.	St.B.	Masd.L.	Lam.	Lam.	Jacou	Vend.	Jacou	Montp.	Montp.	Montp.	Montp.	Montp.	Montp.	Montp.	Montp.	Front.	Front.	Vend.	Montp.	Montp.	Montp.	G.B.B.	Montp.	Montf.	Montf.	Montp.	Montf.	Montp.	Montp.	Front.	Front	Lam.	Lattes	Lattes	Lattes	Lattes	Lattes	Stetigkeit: 50 Aufnahmen Montpellier	30 Aufnahmen St.-Bl. Montpellier	20 Aufnahmen Banyuls
Letzte Düngung	XII·59	'58	III·59	I·59	I·59	'57	III·60	IV·60		'57	II·6	XI·59		III·60	I·60	'56		IX·59	'58	'58	I·59	XII·59	'58	XI·58	'58	'56	'58	'57	I·59	III·59		XII·59	X·58	III·59	III·59	II·60	'59	'57	I·59		X·59	III·59	XII·59	'57	I·60	II·60	[illegible]	[illegible]	II·60	III·59	'56	VI·59			
Letzte Bearbeitung	VII·59	XII·60	X·59	X·59	VIII·59	VIII·59	III·60	1958	X·59	III·60	II·6	II·60		III·60	VII·59			III·60	II·60	II·60	III·60	II·60	X·59	XI·59	X·59	VI·59	X·59	II·60	II·60	I·60	II·60	XII·59	II·60	II·60	III·60	II·60	XI·59?	VI·59	I·60	IX·59	XI·59	II·60	XII·59	III·60	I·60	II·60	[illegible]	[illegible]	[illegible]	[illegible]	II·60	X·59			
Homogen, heterogen	ho	±ho	ho	±ho	ho	ho	ho	he	ho	he	ho	he	±ho	±ho	ho	ho	ho	ho	ho	he	he	he	ho	ho	ho	ho	ho	ho	ho	ho	ho	he	he	ho	ho	ho	±ho	ho	ho	ho	ho	ho	ho	he	ho	he	ho	[illegible]	he	he	ho	ho			
Deckungsgrad %	85	30	30	70	70	20	50	10	60	20	4	10	35	15	15-20	25	70-80	3	5	5	15	3	50	60	40	90	25	5	15	40	25	5-10	30	25	30	30	20-25	90	20	40	40	60	45	15	50	5-10	10	15	5	15	10	70			
Kalkgehalt %	0	0	0	2	5	15	3	0	50	22	30	0	65	55	38	0		0	0	28	0	4	20	40	14	2	10	3,2	20	70	20	5	0	30	40	10	35	16	2	45	0	38	22	7		44	0	50	56	3	15	8			
pH	6,5	7,6	7,5	7,7	7,9	8,0	8,1	7,5	8,0	7,9	8,0	8,1	8,1	8,0	8,1	6,8		6,2	5,9	8,2	7,7	7,9	7,9	8,1	8,0	7.9	8,0	8,0	8,0	8,1	7,9	8,0	7,6	8,0	8,3	8,0	8,1	7,6	7,9	8,1	7,8	8,0	8,1	8,0		8,0	6,7	[illegible]	8,2	8,0	8,0	[illegible]			
Datum Aufnahme 1960	12·IV	12·IV	12·IV	12·IV	7·IV	7·IV	7·IV	16·IV	29·III	29·III	5·I	4·IV	11·IV	2·IV	11·IV	12·IV	10.IV	16·IV	16·IV	31·III	29·III	31·III	2·IV	30·III	2·IV	4.IV	2·IV	4·IV	2·IV	5·IV	6·IV	6·IV	1·IV	8·IV	9·IV	10·IV	28·III	30·III	4·IV	10·IV	4·IV	11·IV	6·IV	29·III	6·IV	6·IV	16·IV	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]			
Datum Kontrolle 1960								2·V	19·IV	19·IV	20·I	20·IV	20·IV	19·IV	22·IV			2·V	2·V	19·IV	19·IV	19·IV	20·IV	20·IV	20·IV	20·IV	20·IV	19·IV	19·IV	21·IV	22·IV	22·IV	19·IV	21·IV	22·IV	22·IV	20·IV	20·IV	22·IV	22·IV	20·IV	22·IV	22·IV	19·IV	22·IV	22·IV	2·V	2·V	[illegible]	[illegible]	[illegible]	21·IV			
Nummer der Aufnahme	38	39	42	41	30	31	29	49	4	2	22	19	8	12	36	40	43	50	52	10	5	11	16	6	14	20	15	17	13	23	25	26	9	32	33	34	1	7	21	35	18	37	24	3	27	28	51	45	44	47	46	48			
Aufnahmefläche 100 m²																																																							
Charakter-Arten der Assoziation																																																							
Diplotaxis erucoidis DC.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+.1	.	.	.	+.1	1.1	+.1	1.1	+.1	1.1	1.1	1.1	.	.	.	1.1	1.1	.	.	1.1	2.1	.	+.1	+.1	1.1	+.1	+.1	1.1	.	+.1	1.1	+	+.1	1.1	.	.	1.2	25	27	–
Aristolochia clematitis L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1.1	.	.	.	.	.	+	.	.	.	.	1.1	.	.	+.1	+	+	+.1	+	.	+	+.1	1.1	1.1	1.1	13	18	–
Veronica persica Poir.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1·2.1	+	.	.	.	.	.	.	.	.	.	.	.	3.3	.	.	.	.	.	.	2.3	.	.	+.1	+.1	.	.	.	.	.	.	+.1	+.1	.	+.1	(+)	10	9	–
Xanthium orientale L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+.1	.	.	.	.	.	.	.	.	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+	.	.	(+)	.	.	.	+	.	6	5	–
Solanum nigrum L. em. Mill.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	1	$\boxed{16}$	2
Verbands-Charakter-Arten (Diplotaxidion)																																																							
Fumaria micrantha Lag.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+.1	.	+.1	.	.	.	.	+	.	.	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	5	2	–
Ordnungs-Charakter-Arten (Chenopodietalia)																																																							
Lamium amplexicaule L.	.	+.1	+.1	+.1	+.1	+.1	+.1	+.1	+.1	1.1	.	+.1	+.1	.	+.1	+.1	+.1	+	.	+.1	.	+.1	+.1	+.1	.	+.1	R	.	.	+.1	.	.	+.1	+.1	+.1	R	+.2	R	+.1	+.1	1.1	.	.	+.1	.	.	(+)	+.1	+.1	+.1	.	[illegible]	[illegible]	[illegible]	[illegible]
Fumaria parviflora Lmk.	.	+.1	.	.	.	.	.	+	+.1	+.1	.	+.1	2·3.1	+	.	+.1	.	.	1.1	.	+.1	(+)	+	1.1	1.1	+.1	+	+	+	.	1.1	1.1	(+)	+.1	+.1	+	+	.	+	.	+.1	.	1.1	+.1	3.2	1.1	.	.	.	.	+.1	.	32	16	7
Carduus tenuiflorus Curt.	.	.	+1	.	.	+.1	.	+.1	.	.	R	+	.	+.1	.	.	.	.	.	(+)	+.1	.	.	.	+.1	+.1	+	+.1	+.1	+.1	+.1	+.1	+.1	(+)	+.1	+.1	+	+	1.1	+.1	.	+.1	.	.	(+)	+.1	.	.	.	+	(+)	+	30	17	1
Fumaria officinalis L. var. densiflora DC.	.	+.1	.	+	1.1	+.1	.	.	+.1	+.1	+.	.	+.1	.	.	+.1	.	.	+.1	.	+	.	.	.	(+)	.	+.1	.	+.1	.	+.1	+.1	.	+.1	+.1	.	+.1	.	.	+.1	+.1	.	+.1	+.1	(+)	1.1	.	.	.	.	.	+	27	[illegible]	7
Rumex pulcher L.	.	+.1	.	+.1	+.1	.	.	+.1	.	.	.	.	1.1	.	.	.	.	.	.	+.1	+.1	+.1	+.1	(+)	1.1	+.1	+.1	+.1	.	1.1	+.1	(+)	+.1	(+)	.	+.1	+.1	.	+.1	+.1	+.1	+.1	.	1.3	.	.	.	.	.	.	.	.	26	[illegible]	6
Stellaria media (L.) Vill.	.	.	.	.	.	.	+	+.1	.	1.3	.	2.3	.	R	1.1	.	.	.	.	+.1	+.1	+.1	.	.	.	.	.	.	+.1	.	.	.	.	.	.	+.1	2.3	2.1	2.1	.	2.1	1.1	.	1.3	.	.	(+)	1.1	+.1	+.1	.	2·3.2	22	30	6
Mercurialis annua L.	.	.	.	.	R	.	.	.	+	+	+.	.	.	.	.	.	.	(+)	.	R	.	.	+	1.1	(+)	.	+.1	(+)	(+)	(+)	.	.	.	+.1	+.1	.	.	1.1	+.1	(+)	.	.	.	.	.	.	.	+.1	.	+.1	.	.	20	15	–
Malva silvestris L.	.	.	.	.	.	.	.	.	.	.	.	+.1	.	.	R.1	.	+	.	.	.	.	(+)	.	+.1	.	+.1	R	+.1	.	.	+	1.3	.	.	.	.	+.1	.	+.1	.	+.1	.	.	(+)	1.1	+.1	+	.	.	.	+.1	+.1	19	20	–
Hordeum murinum L. ssp. leporinum (Link) A. et G.	.	.	.	.	.	.	.	.	.	+	.	(+)	+.2	.	.	.	.	.	.	.	1.2	.	+	.	+	.	.	(+.2)	.	1.2	.	(+)	+.2	.	+	+.2	+.2	.	+.2	+.2	+.2	.	.	(+.1)	(+.2)	.	.	+2	.	.	.	.	$\boxed{19}$	1	1
Galium aparine L.	.	.	.	.	.	.	.	.	.	.	+.	(+)	.	+.1	.	.	.	.	.	.	.	.	+.1	+	R.1	.	+.1	.	.	.	.	1.1	+.1	.	.	+.1	.	1.1	.	(+)	+.1	.	+.1	+.1	.	.	.	+	.	.	.	.	$\boxed{16}$	2	–
Anacyclus clavatus Pers.	.	.	.	.	.	.	.	.	.	.	.	+	+	.	+.1	.	.	.	.	+.1	+	.	.	.	+	.	.	+	.	.	+	.	.	.	.	.	.	+	.	.	+	.	.	+.1	1.1	+	.	.	+	.	+	.	15	14	–
Avena sterilis L.	.	.	.	.	+	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	5	6	–
Urtica urens L.	.	.	.	.	.	.	.	.	.	+.1	.	.	.	.	R	.	.	.	.	.	.	(+)	.	.	.	.	.	.	.	.	.	+	.	.	.	.	1.1	.	.	.	.	.	.	+.1	.	.	.	.	.	+	.	.	7	9	–
Sisymbrium officinale (L.) Scop.	.	.	.	.	.	.	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	5	0	–
Erodium ciconium (L.) L'Herit.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	4	3	–
Diplotaxis tenuifolia (L.) DC.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	4	$\boxed{13}$	–
Klasse Charakter-Arten (Chenopodietea)																																																							
Lolium rigidum Gaud.	3.2	2.2	1.2	2.2	1.2	+.2	1.2	1.2	2·3.2	1.2	1.	.2	1.2	+.2	1.2	+.2	2.2	+.2	1.2	1.2	+.2	1.1	3.2	3.2	2.2	2.2	+.1	1.2	1.2	.	1.1	+.2	1.2	1.2	1.2	1.2	2.2	+.2	1.2	1.2	1.2	3.2	3.2	1.2	1.2	1.2	1.2	+.2	+.1	1.2	+.2	1.2	51	34	7
Sonchus oleraceus L. em. Gouan	+.1	+.1	+.1	+.1	+.1	+.1	+.1	1.1	+.1	+.1	1.	+.1	+.1	+.1	+.1	+.1	+.1	1·2.1	+.1	+.1	1.1	+.1	1.1	1.1	+.1	1.1	+.1	+.1	+.1	1.1	+.1	+.1	.	+.1	+.1	+.1	+.1	+.1	+.1	+.1	+.1	+.1	1.1	+.1	1.1	1.1	+	+.1	+.1	+.1	+.1	+.1	51	36	4
Senecio vulgaris L.	+.1	+.1	+.1	+.1	+.1	+.1	+.1	+.1	+.1	1.1	.	+.1	+.1	.	+.1	+.1	+.1	+	+.1	+.1	+.1	+.1	+.1	1.1	+.1	.	+.1	(+)	+.1	+.1	+.1	+.1	R	+.1	+.1	+.1	+.1	+.1	+.1	1.1	+.1	+.1	1.1	+.1	+.1	(+)	+	+.1	+.1	+.1	(+)	.	48	33	7
Euphorbia helioscopia L.	.	.	1.1	1.1	2.1	+.1	1.1	.	+.1	.	R	1.1	(+)	(+)	+.1	+.1	+.1	.	.	+.1	+.1	.	.	+.1	1.1	1.1	(+)	+	.	.	.	.	.	.	+.1	+.1	(+)	1.1	1.1	2.1	1.1	+.1	(+)	.	.	.	.	.	.	.	+	[illegible]	$\boxed{31}$	16	4
Lactuca scariola L.	.	.	+.1	.	(+)	+.1	+.1	(+.1)	.	+	R	(+)	.	+	+.2	.	.	(+)	.	.	+	.	.	.	.	.	.	.	.	R	.	+.1	.	.	+	+.1	.	.	.	+.1	(+)	.	.	+	.	+	(+)	+.1	+	+.1	+.1	+.1	$\boxed{26}$	4	–
Erigeron canadense L.	+	+.2	1.1	.	.	+	+	.	.	+	.	.	+.1	.	+.1	+.1	.	(+)	.	.	.	+	.	.	.	.	.	+	.	+	+	.	(+)	+	.	1.1	+	.	.	+.1	+	.	.	.	.	.	+	1.1	.	.	.	.	$\boxed{22}$	1	1
Lepidium draba L.	3.2	+.1	+	.	.	.	.	.	.	.	.	.	.	.	+.1	.	+	+	+.1	+	1.1	.	.	.	.	1.1	.	1.1	+	1.1	.	1.1	1.1	+.1	.	+.1	.	.	+.1	+.1	(+)	.	.	.	.	.	.	.	+.1	.	.	.	21	17	1
Sonchus asper (L.) Hill	.	.	+	.	+.1	+.1	+.1	.	.	.	.	.	.	.	+.1	.	+.1	+	.	.	R	.	.	.	.	.	.	.	.	.	+.1	.	.	.	.	+.1	+	.	+.1	.	.	+.1	.	+.1	.	.	+	.	.	.	.	+.1	$\boxed{16}$	7	–
Capsella rubella Reut.	.	.	+.1	.	1.1	.	2.1	.	.	(+)	.	+.1	+.1	.	+.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+.1	.	.	.	.	+.1	+.1	+.1	.	.	.	+.1	.	.	+.1	.	.	.	.	.	.	.	+.1	14	11	–
Veronica polita Fries	.	.	+	+.1	+.1	+.1	.	.	.	(+)	.	.	.	(+)	.	.	+	.	.	.	+.1	+.1	.	1.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+.1	+.1	.	.	+.1	.	.	.	.	+.1	.	.	.	14	10	–
Chenopodium album L.	.	.	+.1	.	.	.	.	.	.	.	.	+	+.1	.	.	.	.	.	.	+	+	+	.	.	.	.	+.1	.	.	.	.	1.1	.	.	.	+.1	.	.	1.1	.	.	.	.	+	.	.	+	.	.	.	+	.	13	$\boxed{35}$	2
Poa annua L.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	4	5	–
Begleiter																																																							
Convolvulus arvensis L.	+.1	.	.	+.1	1.1	.	+.1	1.1	1.1	1.1	1.	.1	+.1	1.1	+.1	+.1	+.1	+	1.1	+.1	+.1	1.1	1.1	+.1	1.1	+.1	1.1	1.1	+.1	1.1	1.1	1.1	+.1	1.1	2.1	1.1	+.1	1.1	1.1	+.1	1.1	+.1	+.1	+.1	2.1	1.1	1.1	+.1	+.1	1.1	1.1	1.1	49	35	18
Lagoseris sancta (L.) K. Maly	+.1	+.1	.	+.1	2.1	.	1.1	+.1	1.1	1.1	+.	+.1	+.1	1.1	.	+.1	+.1	+	+	+.1	1.1	+.1	+.1	1.1	1.1	1.1	+.1	1.1	1.1	+.1	1.1	.	+.1	1.1	+.1	+.1	+.1	3.1	+.1	1.1	1.1	+.1	+.1	+.1	+.1	.	+	+.1	.	+.1	+.1	1.1	46	28	–
Calendula arvensis L.	1.1	.	+.1	+.1	1.1	.	1.1	.	+.1	+.1	+.	+.1	+.1	.	.	+.1	.	.	.	.	.	1.1	+.1	+.1	1.1	1.1	1.1	+	1.1	.	+.1	+.1	.	+.1	+.1	+.1	+.1	+.1	+.1	+.1	+	+.1	.	+.1	1.1	(+)	1.1	.	.	.	+.1	.	35	29	–
Cirsium arvense (L.) Scop.	.	.	+.1	+.1	.	.	(+)	.	1.1	+.1	+.	+.1	.	.	+.1	1.1	1.1	.	.	.	.	.	+	2.1	1.1	1.1	.	1.1	.	+.1	.	.	1.1	1.1	2.1	.	+.1	.	+.1	(+)	1.1	+.1	2.1	1.1	.	(+)	(+)	.	+.1	+	.	+.1	31	27	–
Papaver rhoeas L.	.	+	+.1	+.1	1·2.1	1·2.1	+.1	1.1	+.1	1.1	.	.	1.1	+	.	+	+.1	.	+.1	.	+.1	+.1	.	.	.	.	.	+	+	+.1	.	.	.	+.1	2.1	+.1	+.1	.	+.1	+.1	.	.	.	+	.	.	.	+.1	+.1	.	.	+.1	$\boxed{29}$	6	–
Veronica hederifolia L.	.	.	.	.	1.1	+.1	(+)	+.1	+.1	2.3	.	+.1	+.1	+	.	+.1	.	.	.	+.1	.	.	.	.	.	.	.	(+)	.	.	.	.	1.1	+.1	+.1	.	+.2	.	.	.	2.2	.	.	1.3	1.1	+.1	(+)	+.1	+	+.1	+.1	1.2	$\boxed{26}$	9	–
Rumex crispus L.	.	+	.	.	.	.	.	+.1	.	.	.	+	.	.	1.1	+	1.1	.	.	+.1	+	+	+	+	.	.	+	+	.	+	.	.	+	.	.	.	.	.	.	.	+	.	.	+.1	.	.	+	+.1	+.1	.	+	+.1	$\boxed{22}$	3	–
Crepis taraxifolia Thuill.	.	.	+	.	.	+	.	.	+	.	.	.	.	.	+.1	.	.	+	.	.	+	.	.	.	.	+	+	+	.	.	+	.	+	+	.	+	.	+	.	+.1	+	+.1	.	.	+	.	+	+	+	.	.	.	$\boxed{21}$		–
Geranium rotundifolium L.	.	.	.	.	+.1	+.1	.	(+)	(+)	.	.	(+)	.	.	.	.	.	(+)	.	.	.	.	+	.	.	1.1	.	+.1	(+)	+.1	.	.	+.1	.	.	2.1	+.1	1.1	+.1	+.1	+	+.1	.	.	.	.	.	.	.	.	.	.	19	16	–
Anthemis arvensis L.	+.1	.	.	.	.	.	.	+.1	+.1	+.1	.	.	1.1	1.1	.	.	+.2	+	.	.	.	.	.	.	.	.	.	.	1.1	+	.	.	+.1	.	+	+.1	.	.	1.1	.	+	.	+.1	.	.	.	(+)	.	.	.	+	(+)	$\boxed{19}$	3	–
Cynodon dactylon (L.) Pers.	.	.	.	+	.	.	.	+.1	.	.	+	.	+	.	.	+	.	.	+	+	+	.	+	.	+	.	+	.	+	.	+	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	17	26	–
Muscari neglectum Guss.	+.1	+.1	.	+.1	+.1	+.1	+.1	.	+.1	.	.	.	.	+.1	.	.	.	.	.	.	+.1	+.1	+.1	R	.	.	.	.	.	.	.	.	+.1	R	.	.	.	+.1	(+)	+.1	.	.	.	.	.	.	.	.	.	.	.	.	17	12	–
Medicago orbicularis All.	.	.	.	.	.	.	.	.	+.1	.	.	+	.	.	.	.	.	.	.	.	.	.	+	.	+	2.2	.	.	+	+	+	.	.	.	.	+	+	+.2	+	+	+	+	+	.	.	.	.	.	.	.	.	.	16	14	–
Erodium cicutarium (L.) L'Hérit.	.	.	+	+	+.1	.	1.2	.	.	.	.	.	.	1.1	+	.	.	+	.	(+)	1.1	.	.	+	.	.	.	.	.	.	(+)	.	(+)	.	.	.	.	.	1.1	.	.	1.1	+.1	.	.	.	(+)	.	.	.	.	.	16	11	–
Euphorbia serrata L.	.	.	.	.	.	.	.	.	+.1	+.1	+	.	.	.	.	.	.	.	.	.	+.1	.	.	+.1	.	.	R	+.1	(+)	+.1	+.1	+.1	1.1	.	.	.	.	.	.	.	.	.	+.1	.	1.1	+.1	.	.	.	.	.	.	15	10	–
Reseda phyteuma L.	.	.	.	.	+.1	+.1	(+)	.	+.1	.	.	.	(+)	.	.	.	.	(+)	+.1	.	.	.	.	.	.	1.1	.	.	+.1	.	.	.	.	+	+	.	.	.	.	+.1	.	.	.	.	1.1	+.1	+	.	.	.	.	.	15	6	–
Vicia hybrida L.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	(+)	.	.	.	.	.	+.1	+.1	.	.	+	1.3	(+.1)	+.1	+	.	.	.	3.2	1.1	.	+.1	(+.1)	.	+.1	.	.	.	.	.	.	.	.	$\boxed{15}$	2	–
Vicia angustifolia L.	+	.	.	.	.	.	.	.	(+)	+	.	.	.	.	.	.	.	.	.	(+)	.	.	+.1	+.1	.	.	.	.	.	.	+	.	+	.	.	.	.	+.1	.	.	+	(+.1)	.	(+)	.	.	.	.	.	+	.	.	$\boxed{13}$		–
Medicago minima (L.) Bartal.	.	.	.	.	1.2	.	.	.	+.1	.	.	.	+	.	.	.	.	.	.	.	.	+	.	.	+	2.2	+	.	+	.	.	.	.	+	.	+	.	2.2	.	.	.	.	2.1	.	.	.	+	.	.	.	.	.	$\boxed{13}$	3	–
Erodium malacoides L.	.	.	.	.	.	.	1.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+.1	1.1	1·2.1	+	.	.	1.1	.	+.1	.	.	(+)	.	+	.	1.1	.	+.1	.	.	(+)	.	.	.	(+)	.	.	.	.	.	13	13	–
Centaurea aspera L.	.	.	.	.	.	.	.	.	+	.	.	.	+.1	+.1	.	.	.	.	.	.	+.1	.	.	.	+.1	.	+	+	.	.	.	.	(+)	.	+	.	.	+	+	.	.	.	.	.	+	.	.	.	.	.	.	.	$\boxed{12}$	3	–
Arenaria serpyllifolia L.	.	.	1.1	.	+.1	+.1	+.1	.	+.1	.	.	.	(+)	.	.	.	.	.	.	.	1.2	.	.	.	.	.	.	.	.	+.1	.	.	.	.	.	+.1	.	+.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	$\boxed{11}$	1	–
Torilis nodosa (L.) Gaertn.	.	+	+	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	+	+	.	.	.	.	.	.	.	.	+	.	+	.	+	.	+	.	.	.	.	+	.	.	.	.	.	$\boxed{10}$	2	–
Antirrhinum orontium L.	.	.	.	.	.	.	.	(+)	.	.	.	.	.	.	.	.	.	(+)	.	.	.	.	.	.	R	.	R	.	.	.	.	.	.	+	.	.	.	.	.	(+)	.	.	.	.	+	+	+	.	.	.	.	(+)	10	5	–
Picris echioides L.	.	.	.	.	.	.	.	.	.	+	.	+	.	.	+.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	R	.	.	+	+	+	.	+	.	.	.	.	.	.	.	.	.	.	.	.	8	6	–
Vicia sativa L.	.	.	.	.	.	+	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.	.	+.1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.	.	+	+	.	.	.	.	8	2	–
Crepis pulchra L.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	+	.	.	.	.	+	.	.	.	.	.	.	.	.	.	+	.	.	.	+	+	.	.	.	.	.	(+)	.	.	.	.	.	7		–
Cerastium glomeratum Thuill.	.	.	+	.	+.1	.	.	.	.	.	.	1.1	.	.	.	.	+	.	.	.	+.1	.	.	.	.	.	.	.	.	.	.	.	+.1	.	.	.	.	.	.	1.1	.	.	.	.	.	.	.	.	.	.	.	.	7	2	–
Rubus spec.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	+	.	.	.	.	.	.	+	.	.	.	.	2.1	.	.	+	.	.	.	+	.	.	+	.	.	.	.	.	.	.	.	.	7	9	–
Polygonum aviculare L.	.	.	+	.	.	.	.	+.1	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	.	.	+	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	+	.	.	.	+	.	.	.	.	7	3	–
Geranium molle L.	.	.	.	.	.	.	.	+	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	+	.	.	+	.	.	.	.	.	.	.	+.1	+	.	.	.	.	.	.	.	.	.	.	+	.	+	7	6	–

bestimmtes sagen, zumal auch keine Herbstaufnahmen von meinen Probeflächen vorliegen. Immerhin zeigen die Aufnahmen von Banyuls ein Bild das von denjenigen Montpelliers völlig verschieden ist. Die folgende Tabelle soll dies veranschaulichen.

Diplotaxidetum e. Montpellier Anzahl der Charakterarten		Assoziation Banyuls
der Assoziation	5	1
des Verbandes	1	—
der Ordnung	18	6
der Klasse	11	8

Siehe auch die Gegenüberstellung der Stetigkeit bei den Aufnahmen von Montpellier und Banyuls Tabelle 1.

Wenn man die Aufnahmen der Tabellen vergleicht ist auffallend wie verschiedenartig die Aufnahmen sind. Kaum zwei Aufnahmen sind einander ähnlich. Man darf nicht vergessen, dass die Weinberge ein oder zwei Mal pro Jahr umgearbeitet werden. Nach einer Bearbeitung haben immer neue Samen aus der Umgebung die Möglichkeit sich in diesem "offenen" Gebiet anzusiedeln. Da ausserdem die Zeit der Bearbeitung und insbesondere die Düngung von Weinberg zu Weinberg verschieden sind, ist es nicht verwunderlich dass die Vegetation dieser Weinberge ein äusserst kompliziertes Bild darstellt und das sie viele Pflanzenarten umfasst.

Darf ich am Ende dieser Arbeit Herrn Professor J. Braun-Blanquet und seinem Mitarbeiter Herrn R. Sutter herzlich danken für ihre Hilfe bei diesen Untersuchungen, die ich mit einem Stipendium von der "Koninklijke Nederlandse Akademie van Wetenschappen" in Amsterdam ausgeführt habe.

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Fortsetzung Tabelle 1

Artenverteilung; die Ziffern beziehen sich auf die einzelnen Aufnahmen.

		Stetigkeit 40 Aufnahmen Br.–Bl.
Ordnungs-Charakter-Arten		
2 ×		
Bromus sterilis L.	34, 37	0
Lamium purpureum L.	23, 43	3
Silybum marianum (L.) Gaertn.	18, 23	3
1 ×		
Artemisia verlotorum Lamotte	23	0
Begleiter		
6 ×		
Medicago Gerardi Willd. (M. rigidula Desr.)	4, 7, 13, 20, 21, 25	0
Picris hieracioides L.	7, 34, 35, 43, 45, 48	0
Plantago lanceolatum L.	9, 27, 28, 40, 43, 48	1
Scorpiurus subvillosus L.	5, 13, 18, 24, 35, 3, 7	1
Silene cucubalus Wib.	15, 16, 24, 25, 34, 35	1
Tragopogon australis Jord.	7, 13, 20, 24, 35, 48	0
5 ×		
Anchusa azurea Mill.	5, 9, 16, 26, 43	3
Bromus madritensis L.	1, 2, 7, 15, 23	0
Daucus carota L.	2, 14, 18, 34, 43	4
Galium mollugo L.	1, 5, 6, 9, 15	1
Medicago lupulina L.	8, 37, 47, 50, 51	5
Medicago polymorpha Willd.	1, 6, 16, 19, 23,	0
Ornithogalum umbellatum L.	14, 22, 23, 35, 43	4
Sinapis arvensis L.	10, 32, 36, 43, 49	1
Tordylium maximum L.	4, 7, 16, 34, 46	0
Veronica agrestis L.	5, 30, 31, 32, 35	0
4 ×		
Scorzonera laciniata L.	9, 21, 35, 36	0
Sherardia arvensis L.	8, 9, 31, 39	0
3 ×		
Barbarea vulgaris R.Br.	23, 29, 42,	0
Erophila verna (L.) Chevall.	31, 40, 41	0
Euphorbia portlandica L.	25, 27, 43,	0
Geranium dissectum L.	30, 40, 43,	3
Medicago arabica (L.) Huds.	11, 20, 23	2
Onobrychis viciifolia Scop.	25, 36, 37	0
Lithospermum arvense L.	3, 21, 40,	2
Vicia cracca L.	38, 50, 52,	0

2 ×

Ajuga reptans L.	40, 42,	
Allium polyanthum Borcau	30, 35	**15**
Allium porrum L.	30, 31,	
Allium vineale L.	38, 39,	2
Anagallis arvensis L.	31, 33,	
Campanula erinus L.	35, 50,	
Cnicus benedictus L.	9, 11,	
Cucubalus baccifer L.	44, 48,	
Filago spatulata C. Presl.	30, 31	
Lactuca viminea L.	5, 14	
Lathyrus sativus L.	8, 34,	
Linaria simplex (Willd.) DC.	12, 31	
Melilotus italica (L.) Link.	14, 16,	
Minuartia hybrida (Vill.) Schischk	30, 31,	
Ranunculus sarduus Crantz.	39, 40,	
Rumex conglomeratus Murr.	1, 4,	
Sanguisorba minor L.	9, 31,	
Scabiosa atropurpurea L.	9, 32,	
Silene alba (Mill.) E. H. L. Krause	31, 34,	
Taraxacum officinale Weber	5, 29,	
Tyrimnus leucographus (L.) Cass.	33, 35,	
Urospermum Daleschampsia (L.) Schmidt	17, 25	
Veronica arvensis L.	40, 42,	
Veronica cymbellaria Bodard	7, 23,	

1 ×

Allium sativum	48,
Artemisia annua L.	23
Beta vulgaris L.	45,
Brassica oleracea L.	2,
Bunias ericago L.	41,
Cardamine hirsuta L.	23,
Centaurea collina L.	18
Coronilla scorpioides (L.) Koch	7,
Descurainia sophia (L.) Webb. ex Prantl	49,
Epilobium rosea Schreb.	45,
Filago germanica (L.) L.	31
Fumaria capreolata L.	23,
Galactitis tomentosa Moench	34,
Kentrophyllum lanatum DC.	32,
Lathyrus aphaca L.	40
Lathyrus sphaericus Retz.	41,
Linaria repens (L.) Mill.	6
Medicago sativa L.	31,
Mentha rotundifolia (L.) Huds.	40,
Muscaris comosum (L.) Mill.	35,
Poa trivialis L.	45,
Potentilla anserina L.	43,

Ranunculus arvensis L.	40,
Ranunculus ficaria L.	43,
Rapistrum rugosum L.	43,
Reseda lutea L.	25,
Tordylium maximum L.	51,
Tragopogon pratensis L.	34,
Trifolium stellatum L.	7,
Veronica peregrina L.	1.

Fortsetzung Tabelle 2

Begleiter der Assoziation Chenopodium botrys Br.–Bl. 1936 (?)

Allium roseum	70
Anthemis tinctoria	53
Bromus mollis	71
Clematis flammula	62
Corrigula litoralis	58
Crepis foetida	55
Crepis vesicaris	72
Dactylis glomerata	53
Filago spatulata.	72
Galium parisiense	64
Geranium rotundifolia	71
Hypocharis radicata	66
Koeleria villosa	53
Linaria pelliceriana	70
Muscari comosum	72
Piplatherum coerulescum.	64
Silene alba	69
Sinapis arvensis	71
Sisymbrium officinale	71
Torilis nodosa	55
Trifolium campestre	71
T. sabra	70
T. stellatum	54
T. tomentosum	53
Veronica arvensis	72
Veronica hederifolia	72
Veronica hirsuta	69

BRIEF REPORTS

SOCIETY FOR ECONOMIC BOTANY

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BOOK REVIEWS

OF PUBLICATIONS RELATED TO BOTANICAL WORK IN THE NETHERLANDS

I. S. Zonneveld, De Brabantse Biesbosch. Een studie van bodem en vegetatie van een zoetwatergetijdendelta. (A study of soil and vegetation of a fresh-water tidal delta). Mededelingen van de Stichting voor Bodemkartering. Bodemkundige Studies **4**, and Thesis, Wageningen, 1960. Part A: Summary in English: 210 p. inclusive 154 fig. (graphs, diagrams, maps, charts, photographs) and 25 tables. Part B: Full text in Dutch (mimeographed): 396 p. (without fig.). Part C: Appendices: Vegetation map in colour 1: 10.000; Soil map in colour 1: 16.667; Additional maps; Transsections of the soil (in colour); Transsections of vegetations; Soil analyses; Surveys of algae; Ecological diagram of vegetation units; 6 vegetation tables covering 288 sample plot records.

"De Brabantse Biesbosch" is probably the first doctor's thesis ever presented in the Netherlands in the form of a trilogy. This ambitious aspect of the work is not merely accidental; it is also a symbolic reflection of the huge amount of methodically synthesized documentation and interpretation which is brought together in these three volumes.

The area which forms the subject of this study is a large fresh-water delta with a tidal difference of ca. 2 m, which is unique in temperate Europe, and therefore of considerable scientific interest. As yet, the study of this kind of biotope has been largely neglected, both ecologically and pedologically, in Europe and also elsewhere. The main cause of this neglect may be that these biotopes are very difficult of access, which disfavours an ecological study so detailed as modern science demands. This gap in our knowledge has been filled up in an outstanding way by the present study; this was carried out in the years 1948–1955, but several years of orientating research had preceded it.

The author has combined a full pedological investigation, including soil mapping, with a full synecological research and vegetation mapping; the first attempt of this kind in the Netherlands. In the general part a comparison is made between the results of pedological and vegetation ecological study, and here too a practical application of a combination of the two kinds of study is considered; the geography, history and geomorphology of the area and the utilization of its natural resources are described; and the factors of the environment are analysed. The principal agent, the tidal movement, was carefully studied in its horizontal and vertical components; in the latter three factors play a part, viz. the frequency of the flood, the duration of the flood and the flood height. The last factor has been measured in a large number of places by means of a simple and very ingenious automatic floodtop meter invented by the author.

In the pedological part, sedimentation and texture of the sediments as well as the initial alluvial soil formation are considered and physical ripening and shrinkage as well as chemical ripening are dealt with. Special attention is given to the process of decalcification and it is pointed out that this is favoured by a high degree of

humidity which favours anaerobic life and accordingly reduction. Here a first attempt is made to study the initial alluvial soil formation as a pedological process and not merely as a geological or a geomorphological one. The soil-mapping criteria have not been chosen among the instable ones (soil-water relationship), but are found in the more stable physical features, such as the thickness of the clayey and silty topsoil by which the sandy subsoil is covered. In case of a soil which has not yet reached the final stage of its development, the soil profile is considered as it would appear when the shrinkage is completed, and this enables an easy comparison of soil lying inside and outside the dikes. This part is concluded by a consideration of the systematic soil classification and by a study of the geology, genesis and palynology of the area.

The vegetational and ecological part starts with a detailed description of the vegetation units. Vegetation analysis has been carried out with the Braun–Blanquet method but the author has avoided the pitfall of prematurely fitting his units into the syntaxonomic system of the French–Swiss School. The plant communities are described and deliminated as objectively as possible. Though the classification was based on ecological, topographic and physiognomic features, the diagnostic description of each plant community is purely synmorphological (mainly floristical), which is an essential condition for determining the relation between vegetation and habitat. The major classification is as follows: vegetation of 1. permanently submerged parts, 2. rough herbage, 3. reed-marshes, 4. tidal woods and willow coppices, 5. floating mats. Within groups 1–4 a first dubdivision was made according to differences in habitat of a geomorphological kind (low banks, stream levees, back swamps, etc.), and a second subdivision according to the elevation and the influence of the resulting submergence. In an admirable diagram in which all these criteria are combined all vegetation units are shown schematically in relation to the environment. Beside phanerogams, *Chlorophyceae*, *Cyanophyceae* and *Diatomae* are considered; for many species of the genus *Vaucheria* the freshwater tidal delta proved to be the most suitable habitat.

The vegetation has been mapped on the base of these units. All vegetation records (qualitative as well as quantitative structural analyses) were used in dressing the community tables. The author did not make a selection for the sake of a clear representation of vegetation types, all transitions being included in the tables. The vegetation map with its ecological diagram is the most detailed ecological map ever published in the Netherlands.

Study of the succession has been carried out directly (by permanent quadrat plots as well as by interpretation of air photographs yearly taken from an electricity pilon) and indirectly.

Much attention has been given to synecology. The synecological investigations were carried out by measuring in as many places as possible the principal habitat factors prevailing in the different communities (mesological synecology) and by studying how far the aspect of the vegetation reacts to the various environmental factors. Not only the tidal movement (see above), but also the aeration condition and the soil consistency were determined by simple and efficacious methods which enabled the accumulation of a large number of data in spite of the difficulty of access of the habitat. However, an important part of the latter research, viz. the investigation of the phenotypical reaction of the plant species and of individual plants, should not be considered synecology, as the author did, but in fact an important contribution to the autecology of plants growing in the freshwater tidal

delta, so e.g. the study of scleromorphism, of the "sinker effect", of propagation, of aerial root formation. Very interesting results were obtained by the use of life-form spectra both in the sense of Raunkiaer and in that of Iversen; the relation between vegetational scleromorphism and the mechanical influence of the current as well as of relative drought was shown, and so was the relation between the number of telmatophytes and the rate of reduction in the soil.

This part is concluded by a comparison of the vegetation of the Biesbosch with that of other areas, and by a discussion of the syntaxonomic classification of the plant communities according to the system of the "French–Swiss School". This valuable attempt is the more admirable since it is clear that this kind of research has not the author's sympathy and is not his best point. Since describing new syntaxonomic units is not part of the author's ambition, he modestly restricted himself to the description of a single new association, viz. the *Scirpetum triquetri et maritimi*, regarding the other specific communities of the freshwater tidal delta either as subassociations or as variants or, if necessary, describing them as dominance communities (sociations). The syntaxonomic value of these units still has to be determined.

The last part of the work deals with the application of the results of the soil and the vegetation study, as well for the growing of reed, and of willows and other trees as for the development of arable and grassland soils, and last but not least with the significance which the results may have for the reconstruction of the natural landscapes in the Netherlands and their soil-forming processes.

The study is, of course, not perfect. Several points may be criticized. The theoretical considerations which serve as a background for vegetation analyses are not very clear; the author's opinion that one exclusive "faithful species" can be used in vegetation mapping (and not selective and preferent ones) is irrelevant, since vegetation mapping does not consider faithful species (as such) at all, but is based on an diagnosis by means of constant differential species.

A certain carelessness of the author appears from the rather scarce and not always correct quotation of references in the general and methodological part. It is obvious, however, that this nonchalance is only superficial, and that it did not extend to the investigation itself. The defects of the work are negligible in comparison to the very important contribution to science, which is the more admirable when the strenuous character of the field work in this area is considered.

V. Westhoff

J. H. A. Boerboom, De plantengemeenschappen van de Wassenaarse duinen (The plant communities of the Wassenaar dunes near The Hague). (Met bijlage: Vegetatiekaart van het wingebied van de Duinwaterleiding van 's-Gravenhage). Meded. Landbouwhogeschool Wageningen 60(10): 1–135. 1960; Belmontia II. Ecology, fasc. 7. 1961; and Thesis, Wageningen, 1960.

Die ursprünglich so abwechslungsreiche, aber in den letzten 100 Jahren stark verarmte niederländische Landschaft weist heute noch einige Aspekte von hervorragender biologischer Bedeutung auf. Die Dünen nehmen dabei eine besondere Stellung ein wegen ihrer grossen Ausgedehntheit und durch das Vorkommen verschiedener Assoziationen, welche in den Niederlanden ihre optimale Entfaltung erreichen, manchmal sogar fast endemisch sind. Obwohl seit Dodonaeus eine

ununterbrochene Reihe hervorragender Botaniker die Pflanzenwelt dieser Dünen studiert haben, beschränkten vegetationskundliche Gebietsbeschreibungen aus den Dünen sich bisher auf verhältnismässig kalkarme Gebiete und hauptsächlich auf Inseln (Westfriesische Inseln, Bergen, Goeree).

Das vorliegende Buch über ein rund 20 km² grosses, ziemlich kalkreiches Dünengebiet nördlich von Den Haag ist die erste "kontinentale" Dünenstudie von grösserem Umfang, welche nach modernsten pflanzensoziologischen Methoden zusammengestellt wurde. Sie gibt eine vollständige und wohlüberdachte Übersicht der heute vorhandenen Assoziationen, eingeteilt in:

I. Gesellschaften der offenen und trockenen Dünen (Moos- und Flechten-, Gras- und Krautgesellschaften auf grundwasserfernen Böden).
II. Hydrophile Gesellschaften.
III. Strauch- und Zwergstrauchgesellschaften.
IV. Wälder.
V. Unkrautgesellschaften.

Zusammen mit den in der ausführlichen Literaturübersicht genannten, schon eher erschienenen Dünenarbeiten desselben Autors und seiner Mitarbeiter erhalten wir damit ein sehr zuverlässiges Tatsachenmaterial über Vegetation und Umwelt dieses Dünengebiets. Besonders die Abteilungen über Moos- und Flechten-, Zwergstrauch- und Unkrautgesellschaften enthalten Vieles, das grosse Erfahrung und genaue Beobachtungsgabe verrät.

Es ist aber die Aufgabe des Kritikers, auch auf Unvollkommenheiten hinzuweisen. Die Arbeit zeigt Lücken und enthält einige Schönheitsfehler, welche zum Teil vermieden hätten werden können. Vegetationsaufnahmen bzw. Tabellen wurden nur aufgenommen "als Ergänzung und Erläuterung des Textes". Dadurch lässt die Arbeit sich recht gut lesen, wurde den Fachgenossen aber gerade dasjenige Material vorenthalten, welches sie an erster Stelle verlangen durften. Kontrolle der Treuegrade der Kenn- und Trennarten und Kenntnis der vollständigen floristischen Zusammensetzung der verschiedenen Einheiten wird dadurch verwehrt. Die vorhandenen Tabellen enthalten so wenig Aufnahmen, dass Errechnung von Stetigkeitsprozenten und mittleren Artmächtigkeiten wenig Zweck hätte. Vergleichende Übersichtstabellen oder Artenlisten fehlen. Von mehreren Assoziationen fehlen sogar jegliche Aufnahmen. Die Unterschiede zwischen den verschiedenen Dünengebieten sind so wichtig, dass auch bei schon mehrmals beschriebenen Assoziationen diese Grundlagen nicht einfach hätten fortbleiben dürfen. Aus verschiedenen Äusserungen des Autors geht hervor, dass diese Behandlungsweise nicht an erster Stelle wegen den hohen Druckkosten von Tabellen so gewählt wurde.

Hat die Arbeit also einerseits nicht ganz den Charakter einer Gebietsmonographie, so fehlt andererseits doch auch das Material zu einer systematischen Übersicht der Dünengesellschaften. Auf systematische Angaben über Wuchs- und Lebensformen, pflanzengeographische Elemente, Betrachtungen über die höheren Einheiten (Ordnungen, Klassen), sowie auf Vergleich mit den Dünengebieten zwischen Noordwijk und Bergen wurde grösstenteils verzichtet. Die neue Einteilung des Verbandes "*Koelerion albescentis*" in einen Unterverband mit vorwiegend Therophyta, Bryochamaephyta und Chamaephyta lichenosa, und einen Unterverband mit vorwiegend Hemicryptophyta hat viele Vorteile. Die Nahme "*Erodio-Koelerion*" für den erstgenannten Unterverband ist aber abzulehnen. Nachdem die dazu

gehörige Assoziation von *Tortula ruralis* und *Phleum arenarium* etwas voreilig umgetauft wurde (p.p.) in "*Tortula ruralis – Erodium glutinosum*-Assoziation" als Gebietsassoziation des kalkreichen, nordatlantischen pflanzengeographischen "Dünendistrikts", wurde derselbe *Erodium* — beschränkt also auf eine Gebietsassoziation — gewählt für die Namengebung des Unterverbands, welcher gerade die verschiedenen Gebietsassoziationen umfassen soll. Richtig wäre hier: "*Tortulo-Koelerion*".

Dass über die systematische Einteilung mancher beschriebenen Gesellschaften das letzte Wort noch nicht gesprochen wurde, braucht nicht als ein Fehler angemerkt zu werden, obwohl auch hier der Autor sich manchmal recht wenig bemüht hat, die getroffene Wahl zu dokumentieren und zu verteidigen. Falsch ist aber die Rangierung der Aufnahmen der Tab. 17 ("*Convallaria majalis – Quercus robur*-Ass.") in das *Quercion robori-petraeae*, da in dieser Tabelle die *Querco-Fagetea*- und *Alno-Ulmion*-Arten die *Quercion*-Arten in Anzahl und Menge weit überbieten. Nach der Meinung des Rezensenten sollte man diese Bestände, eventuell zusammen mit denen der "*Crataegus monogyna – Betula pubescens*-Assoziation" als besondere Sub-Assoziation des *Fraxino-Ulmetum* werten. Damit ergibt sich aber das Problem der Systematik der Birkenwälder, dessen Erörterung hier zu weit führen würde.

Die beigefügte gedruckte Vegetationskarte, ausgeführt im Maszstab 1:5000 und in gut gewählten Farben, dient als Grundlage bei der Verwaltung des Gebiets, und wurde an anderer Stelle als in den kurzen Angaben im Text der vorliegenden Arbeit ausführlicher erläutert. Sie bedeutet ein überaus wertvolles Stück "angewandte Pflanzensoziologie".

Wir hoffen, dass der Autor in seiner neuen, tropischen Umwelt Gelegenheit finden wird, seine vielen unveröffentlichten Beobachtungen noch weiter auszuarbeiten.

H. Doing

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THE UPTAKE OF CATIONS BY VALLISNERIA LEAVES

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(received August 22nd, 1961)

CONTENTS

CHAPTER I

INTRODUCTION

1. Statement of the Problem

When a plant is transferred from water to a salt solution, there is a short period of rapid uptake of ions, which is followed by a slower uptake. It is generally accepted that the slow uptake depends on metabolism and that the ions are mainly accumulated in the vacuole, whereas the initial rapid uptake is mainly non-metabolic. Characteristics of uptake that depend on metabolism are that it is inhibited by low oxygen tensions, by low temperatures and by certain poisons. Opinions differ as to the question which role is played by metabolism in the initial rapid uptake. The notion of a Free Space, that is, a compartment of the cell which is freely accessible for ions by diffusion, implies that part of the initial uptake is non-metabolic. Because it was not certain that, in this space, ions are actually free Hope and Stevens (1952) introduced the term Apparent Free Space (A.F.S.). Briggs and Robertson (1957) divided the A.F.S. in a "Water Free Space" and a "Donnan FreeSp ace". Ions would move freely in the W.F.S., but in the Donnan Free Space they would be held by coulomb forces. Usually, the amount of cations taken up during the initial rapid diffusion exceeds the number of anions, due to an exchange of cations of the D.F.S. for cations from the outer solutions.

One of the many theories about the mechanism of ion absorption by living cells is the carrier theory. This theory starts from the hypothesis that the ions are bound to some movable compound of the cytoplasm, which can permeate through a membrane that is impermeable for free ions. In this way ions might be absorbed by the living cell.

Van den Honert (1933, 1936) belonged with Stiles (1924) and Lundegårdh (1932) to the first, who supported the theory, that uptake of ions starts with adsorption to a cytoplasmic compound. He showed that, in phosphate absorption by sugar cane, the absorption rate-concentration curves resemble Freundlich's adsorption isotherm. For this reason Van den Honert assumed that the first step in the uptake was adsorption of the phosphate ions. The second phase of uptake would be translocation of the adsorbed ions into the cell. This phase is temperature-sensitive, and, hence, Van den Honert concluded that it depends upon metabolism. He compared the uptake mechanism to a conveyer belt with a constant loading capacity and a rotation velocity regulated by metabolism.

Osterhout (1936), Hoagland and Broyer (1936) and Arisz (1944, 1945) also pointed to the significance of adsorption processes for the absorption of compounds by the cytoplasm Jacobson *et al.* (1950) and Overstreet *et al.* (1952) developed this hypothesis further by advancing the possibility that ions might react with metabolically formed cytoplasmic compounds under delivery of

hydrogen and hydroxyl ions. The complexes formed would be decomposed by a chemical change after passing the barrier.

EPSTEIN and HAGEN (1952), though not starting from a delivery of hydrogen and hydroxyl ions, based their experiments also on the hypothesis that the absorption process implies the formation and breakdown of a complex of an ion and a metabolically formed carrier. They found that quantitatively the absorption of alkali cations is in accordance with the view that a binding in the form of a labile complex occurs in the absorption process.

The relationship can be expressed by the equations

$$R + M \text{ outside} \underset{K_2}{\overset{K_1}{\rightleftarrows}} MR$$

$$MR \underset{K_4}{\overset{K_3}{\rightleftarrows}} R + M \text{ inside}$$

Moreover their experiments point to the existence of specific binding sites each preferring a special ion.

HELDER (1952) also showed that, with intact maize plants, the relationship between concentration and absorption of ions could be represented by an adsorption-isotherm (a Freundlich or a Langmuir curve). As it is known of many enzymatic processes that the relationship between reaction velocity and the concentration of the substrate can also be expressed by a Langmuir equation, he suggests that the first step of the absorption of phosphate is enzymatic.

It has been ascertained for several plant tissues, which are able to absorb ions, that they bind ions which can be removed again by exchange for other ions. This holds especially for the cations. (DEVEAUX, 1896; MAZÍA, 1938; BROOKS, 1938, 1939; STEWARD and HARRYSON, 1939; BUTLER, 1953; EPSTEIN, 1954; BRIGGS, HOPE and PITMAN, 1958; DAINTY and HOPE, 1959). This process of reversible binding of ions is identified by many investigators with the binding to cytoplasmatic compounds according to the carrier hypothesis. Also when they are not inclined to identify the exchange-sites with carriers, these investigators consider the binding to the exchange-sites a necessary step in the absorption of ions. (JACOBSON, 1950; SUTCLIFFE, 1954; HIGINBOTHAM and HANSON, 1955; RUSSELL and AYLAND, 1955; LUNDEGÅRDH, 1958; GONZALES and JENNY, 1958).

Contrary to this view, is the opinion of EPSTEIN and LEGGETT (1954), LATIES (1959) and LAGERWERFF (1961), who attribute little or no value to the exchangeably bound ions for the absorption process.

The aim of the present research was to try, at least with *Vallisneria* leaves, to obtain decisive information about the significance of the exchangeably bound ion fraction for the absorption process.

2. LITERATURE

According to GONZALEZ and JENNY (1958) roots of Alfalfa seedlings absorb cations by surface migration. They demonstrated that Sr ions

which were bound by a cation exchange resin, could be absorbed by roots which were in contact with the resin.

It is true that these experiments proved that cation absorption by plant cells was possible by means of exchange, but it was not proved that under different conditions the exchange step is necessary for the absorption of ions. Moreover it was not demonstrated that the Sr ions, once absorbed, could not be exchanged for other cations. Absorption, in their experiments, may have consisted for one part of an irreversible and metabolically regulated uptake and for the other part of a reversible adsorption.

Higinbotham and Hanson (1955) studying the absorption of rubidium by discs of potato tissue found the same relationship between initial uptake (adsorption exchange) and the concentration of the external solution as between accumulation and that concentration. The curve representing this relationship resembled Freundlich's adsorption isotherm.

They considered this to be an indication that the adsorption exchange is the first, and hence a necessary step in the process of ion uptake. The accumulation would depend on the adsorption exchange fraction, and the latter would depend on the concentration of the outer solution. It will be clear that the fact that initial uptake and the accumulation process bear the same relationship with the concentration of the outer solution does not prove that the initial uptake is a necessary step in the uptake process. The similarity of this relationship in both processes may be a mere coincidence.

Lundegårdh (1958) studying the uptake of potassium and chloride by wheat roots also found a similar relation between initial absorption and concentration of the outer solution and between the accumulation rate and that concentration. He too concluded that the initial absorption limits the accumulation rate. (According to Lundegårdh adsorption exchange is the principal mechanism of the initial absorption). In Lundegårdh's experiments as in earlier investigations, the accumulation started slowly and it only reached its maximal rate after 30 minutes. However, the initial uptake reached 40–80 % of its maximal value after 5 minutes and its maximum within 15 minutes. This makes it improbable that the initial uptake is a necessary phase of the accumulation process. Contrary to Lundegårdh's results Epstein (1954) and Winter (unpublished) found that the accumulation rate attains a constant value within 5 minutes from the start of the experiment.

Scott Russell and Ayland (1955) assume that the rate of the initial rapid uptake is determined by exchange reaction's since the uptake of rubidium was reduced in the presence of other ions.

They conclude that, if adsorption to carriers exists in the sense of Jacobson and Overstreet (1947), it will take place after the initial entry of the cations by exchange. To this the objection may be made that the fact that exchange reactions are rate-determining in the initial rapid uptake does not prove that the same reactions determine the rate of the subsequent slow accumulation process.

Many soil scientists assume that the uptake of cations by roots and the selectivity of this uptake are determined by the exchange capacity of the root system. Often these conclusions are invalidated by the fact that exchangeable cations and cations that are irreversibly accumulated have not been determined separately in their experiments (e.g. Elgabaly and Wiklander, 1949).

3. Material and Methods

The experimental plant, *Vallisneria spiralis*, was cultivated in concrete basins of 110 × 110 × 50 cm which stood in the basement of the laboratory. The roots grew in a 10 cm layer of clay on top of which a thin layer of sand had been placed to prevent the water from being polluted by clay particles. The basins were to the edge filled with demineralized water. The plants were illuminated by means of a Philips 450 Watts mercury vapourlamp for 15 hours daily. The temperature of the water oscillated between 22° C and 24° C.

For uptake experiments the leaves were cut into 2,5 cm segments. The edges were trimmed off leaving a strip of 2,5 × 4 mm. The strips were randomized and divided into sets of eight. Each set was mounted in a small perspex frame (Fig. 1).

The capacity for ion uptake of leaf-strips in light increases during the first hours after cutting, due to recovery from wounding. (Arisz, 1948, 1957; Sol, 1958). For that reason the strips were pretreated in demineralized water for 24 hours. During pretreatment the strips were illuminated by means of an incandescent bulb of 100 Watts at a distance of 50 cm. The water was constantly aerated with air that had been freed from carbon dioxide. The temperature was kept constant at 25° C.

For the determination of ion-uptake two sets were placed in a perspex vessel with parallel walls (Fig. 1). The vessel contained

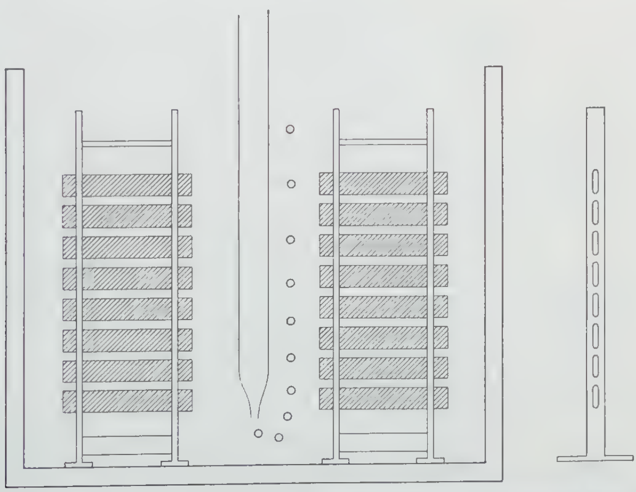

Fig. 1. Two sets of leafstrips in a planparallel perspex container of 150 cc capacity.

125 ml solution. Conditions during uptake were the same as during the pretreatment.

Uptake was determined by means of tracer methods. Rubidium, calcium, chloride and sulphate were labelled with Rb–86, Ca–45, Cl–36 and S–35.

The leaf strips were digested in 2 ml of concentrated nitric acid on a water-bath at 96° C for 2–3 hours. If chloride had to be determined, silver nitrate was added to the nitric acid to prevent the loss of hydrochloric acid during digestion. When the digestion was completed the liquid was made up to a volume of 25 ml with water. Rubidium and chloride were determined in 10 ml of the extract by means of a dipping Geiger–Müller tube. A control solution of known specific activity was counted in the same way. Uptake was computed from the difference of the two countings.

For the determination of sulphate uptake the leaf-strips were not digested but boiled in inactive K_2SO_4 (250 μ mol.). The active sulphate ions in the leaves are exchanged for inactive ions.

It is possible to obtain a 10 percent higher yield of active sulphate from the leaves by ashing with Na_2CO_3 and Na_2O_2 (PAECH, 1955).

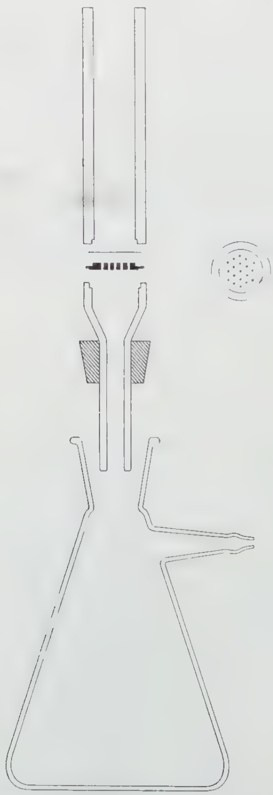

Fig. 2. Apparatus for collecting Ca-oxalate precipitates.

Since differences in uptake only were important the less cumbersome extraction by exchange has been preferred. After extraction the sulphate solution was acidified and the sulphate was precipitated as $BaSO_4$. The precipitate was collected on a filter (Schleicher and Schüll, Blue band, nr. 589/3). The filter was ashed in an oven at 620° C. The barium sulphate was suspended in 96 % ethyl alcohol and transferred to an evaporation dish. After evaporation of the alcohol under an infra-red lamp a layer was obtained which contained 12,86 mg/cm². Its activity was measured by means of a window-less flow counter. Uptake was measured from the difference in counts per minute between the sample and a sample of known specific activity that had been prepared in a similar way.

Calcium was precipitated by adding potassium oxalate after addition of 6,54 mg of calcium chloride to the digest and neutralisation with 20-25 % ammonia, (Iljin, 1939). The added calcium served as a carrier for the radio-active ions. The precipitate was collected on a Schleicher and Schüll Blueband filter, nr. 589/3 (Fig. 2). After drying by means of an infra-red lamp an even layer remained which contained 2,43 mg/cm². The activity was determined by means of an end-window Geiger–Müller tube. Uptake was computed from the difference of this counting and that of a similarly treated precipitate of known specific activity.

CHAPTER II

PRELIMINARY EXPERIMENTS

1. Uptake of Rubidium

As was mentioned already in the introduction, Epstein and Leggett (1954) succeeded in separating the Sr absorbed by excised barley roots into three fractions namely:

a fraction which can be washed out in deionised water.
a fraction which can be removed by exchange.
a fraction which can neither be washed out nor exchanged.

However, with the monovalent cations they did not find an exchangeable fraction. In the present experiments the uptake of monovalent cations by *Vallisneria* leaves could be separated into the same fractions as found by Epstein and Leggett for the uptake of strontium by excised barley roots (Fig. 3). For convenience these fractions were called A, B and C.

In the experiments of Fig. 3 the cation Rb was taken up from a .008 M Rb*Cl solution labelled with the radioactive isotope Rb–86. After an uptake period of 60, 120 and 240 minutes a part of the sets was blotted between double-folded filterpaper, another part rinsed in deionised water for one hour and the rest of the sets was bathed in a .008 M solution of unlabelled RbCl for one hour. In this way it was possible to determine fraction A, B and C.

Fraction A is the difference in Rb* content of the leafstrips which

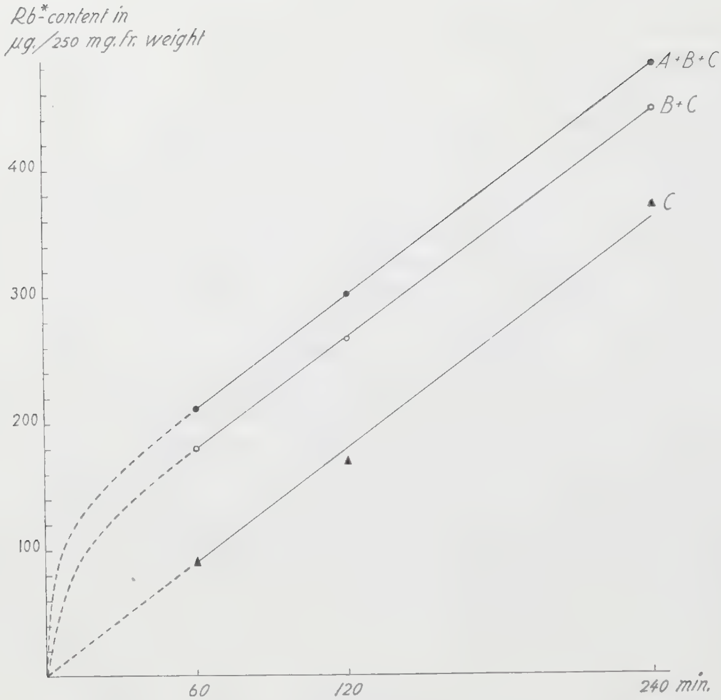

Fig. 3. Rubidium content of leafstrips after absorption from a .008 M Rb*Cl solution for various periods. Treatment of the leaves after removal from the Rb*Cl solution: Blotted (A + B + C); in water for one hour (B + C); in unlabelled RbCl for one hour (C). Each point represents the average value of two replications.

were blotted between double-folded filterpaper and the leafstrips which were rinsed in deionised water for one hour.

Fraction B is the difference in Rb* content of the leafstrips which were rinsed for one hour in deionised water and the leafstrips which were bathed for one hour in a solution of unlabelled RbCl. It contains the exchangeable ions.

Finally fraction C is the quantity of Rb* which remains in the leafstrips after bathing them in a solution of unlabelled RbCl. Fig. 3 shows that fraction C is linearly proportional to time but that on the other hand the fraction A as well as fraction B rapidly reach their maximum value. This value is reached within 60 minutes. The points in Fig. 3 are the averages of two sets. Within a single experiment replicates did not differ more than 5–10 percent.

2. Uptake of Chloride and Sulphate

Vallisneria leaves were bathed in a KCl* solution .008 M labelled with Cl–36. After a sixty minutes uptake period the fraction A, B and C were determined as has been described for the Rb ion.

Fig. 4 shows the Cl uptake. This figure gives the results of three different experiments a, b and c. The length of the columns gives the content of labelled chloride of the leaves at the end of the experiments. The shaded column gives the sum of the fraction A, B and C. The white column the sum of the fractions B and C and the black column gives the fraction C. In Fig. 4a and b there is a

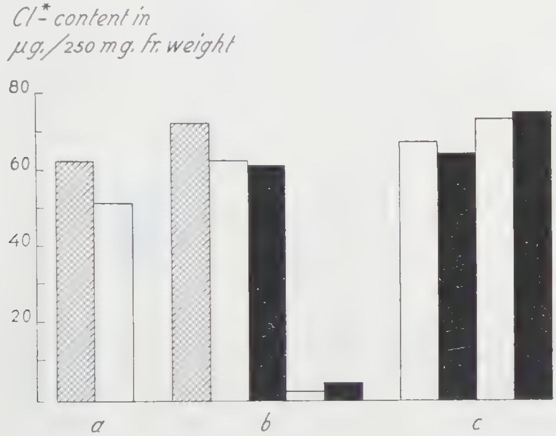

Fig. 4. One hour Cl* uptake from a .008 M KCl* solution. Shaded: fraction (A + B + C); white: fraction (B + C); black: fraction C. Each column represents the average value of two replications; a, b and c are 3 different experiments.

significant difference between the shaded column and the white column indicating that for chloride a fraction A exists. On the contrary there is no significant difference in length of the white and the black column in Fig. 4b and c. Thus if for chloride a fraction B exists it is very small as compared to the fraction B for cations. Because fraction B is determined as a difference, its absolute error is smaller when the values, from which it is determined, are low than when these values are high. For this reason fraction B was determined at 5° C. The two last columns of Fig. 4b give the result. Fraction B was even negative, but the value is not significant. The results with sulphate are shown in Fig. 5.

Owing to the fact that the specific activity of the sulphate solutions was higher than that of the chloride solutions, differences in uptake of sulphate could be determined with a higher accuracy than the differences in chloride absorption. With chloride a fraction B could only be determined if it was of the order of a few μg/250 mg fresh weight, with sulphate the smallest quantity was as low as 0,1 μg/250mg fresh weight. Fig. 5 shows that for sulphate ions a fraction B could not be detected. Sulphate was taken up from a .008 M solution. Each column in Fig. 5 is an average of 4 sets, representing one gram of leaves fresh weight.

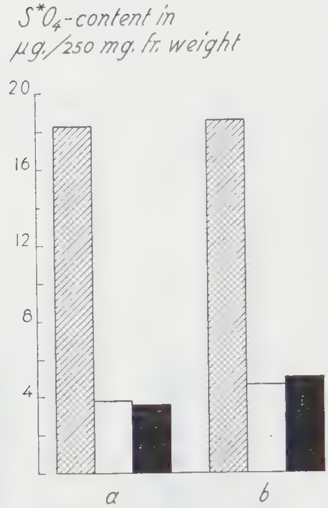

Fig. 5. One hour S^*O_4 uptake from a .008 M $K_2S^*O_4$ solution. Shaded: fraction (A + B + C); white: fraction (B + C); black: fraction (C). Each column represents the average value of 4 replications; a and b are two different experiments.

CHAPTER III

THE DEPENDENCY OF THE Rb FRACTION B ON METABOLIC ACTIVITY

In the literature on ion-uptake metabolic absorption and exchange-absorption are generally distinguished.

Epstein and Leggett (1954) have summarized the properties of these two modes of uptake as follows:

	Exchange adsorption	*Active transport*
1	Non linear with time; equilibrium approached in 30 min.	Linear with time; no equilibrium reached in the experiment.
2	Ions readily exchangeable.	Ions essentially non exchangeable.
3	Not selective with respect to various ions.	Selective with respect to various ions and groups of ions.
4	Requires no energy expenditure on the part of the tissue.	Requires energy expenditure.

In the previous chapter it was shown that the fraction B is readily exchangeable as opposed to the fraction C which is given off neither to water nor to a salt solution. Loss of measurable amounts from the fraction C to the surrounding solution only happens when the condition of the *Vallisneria* leaves declines. A very sensitive indication of less favourable condition is the infiltration of the intercellular spaces with liquid. When the *Vallisneria* leaves are in a good condition the septa in these spaces are permeable to gases but not to water (Solereder, 1913).

The experiments of this chapter were carried out to check if our fraction B conforms to Epstein and Leggett's requirements for exchangeadsorption.

1. The rate of establishment of the equilibrium

A second point of difference between exchange adsorption and active transport is the rate at which equilibrium is established.

Fig. 6 and Fig. 7 show the dependency of fraction B and C on time. After immersing the *Vallisneria* leafstrips for different times in a RbCl solution, fraction B and fraction C were determined as described in the section on methods.

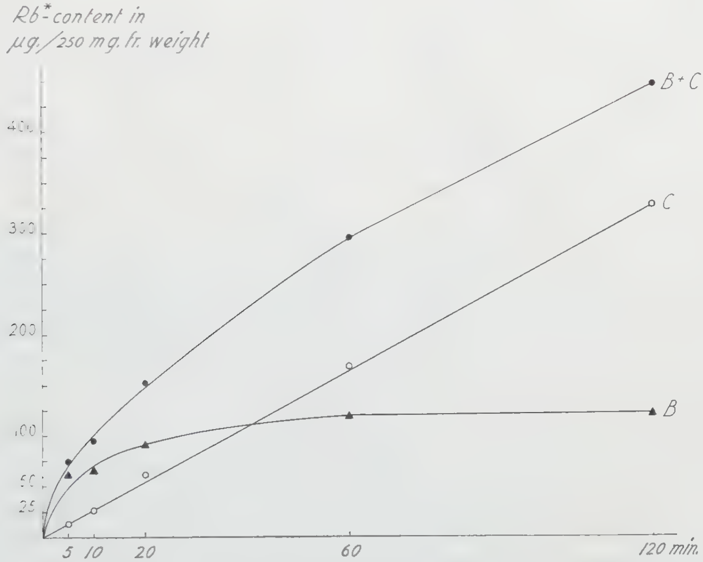

Fig. 6. Course of Rb* uptake from a .008 M Rb*Cl solution. B and C refer to the fraction B and fraction C. Each point represents the average value of two replications.

The uptake of Rb-ions into the fraction C appeared to be constant during the first hour of the experiment (Fig. 6). Under favourable circumstances such constant rate could be maintained during several hours (Fig. 7). We can also see that in *Vallisneria* leaves there exists no significant lag period in the uptake of Rb-ions into fraction C as was found by Lundegårdh (1958) with wheat roots.

The uptake of Rb-ions into fraction B is not linear with time. The rate of uptake during the first 5 minutes is much greater than the rate of uptake into fraction C in the same period, but it decreases very quickly. We can safely assume this rate to be zero after a sixty minute period, and the equilibrium to be established. In the greater part of the experiments equilibrium was found to be established within 30 or 60 minutes. As the amount of material I had at my

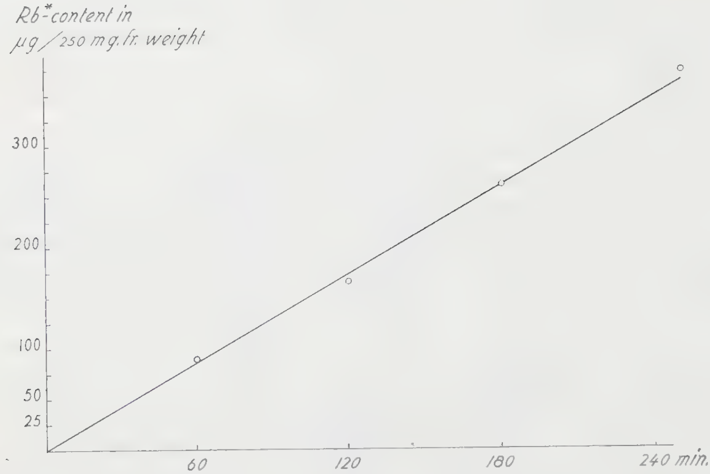

Fig. 7. Course of Rb* uptake into fraction C from a .008 M Rb*Cl solution. Each point represents the average value of two replications.

disposal was limited, the experimental error was too large to determine this time more accurately. The variability among different experiments may be due to the various kinds of *Vallisneria* material used in these experiments. However, the period required to reach equilibrium is of the same order as found with other types of material by other investigators (Laties, 1959; table 1).

2. The effect of temperature

The effect of temperature on physico-chemical processes is much smaller than on metabolic processes. (Wanner, 1948; Höber, 1945). A small effect of temperature on the rate of Rb uptake into fraction B would therefore be a reason to consider this uptake as a physico-chemical process.

Fig. 8 shows the effect of temperature. The uptake proceeded in the dark at 25° C and 5° C for 60 minutes and 30 minutes. In other experiments it was found that the rate at which Rb ions are accumulated in fraction C is much smaller in the dark than in the light. On the other hand fraction B is not influenced by light or dark. If uptake is studied in the light, fraction C is large and an error in its determination will effect the difference between (B + C) and C (i.e. fraction B) much more than when fraction C is small. Therefore, the advantage of studying the uptake in the dark is that the size of the fraction B can be determined more precisely, for the fraction B is determined by measuring the difference between the fractions (B + C) and C. After 60 minutes or after 30 minutes uptake hardly a significant effect of temperature on the rate of uptake of Rb-ions into fraction B could be shown. A 60 minutes uptake period is more than sufficient for the fraction B to reach its maximum value at a temperature of 25° C and, therefore, an effect of temperature might

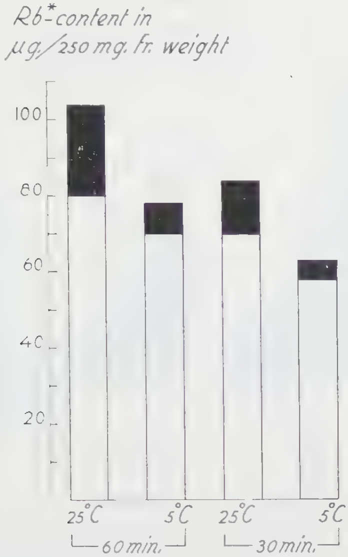

Fig. 8. Rb* uptake in the dark from a .01 M Rb*Cl solution for 60 or 30 minutes and at 25° C or 5° C. Black: Rb* fraction C; white: Rb* fraction B. Each column represents the average value of two replications.

not show up. After 30 minutes of uptake the fraction B is not yet maximal. The fact that temperature has no effect during this period indicates that the effect of temperature on the rate of uptake of Rb-ions into fraction B is small, contrary to the effect of temperature on the rate of uptake of Rb-ions into fraction C.

3. The effect of metabolic inhibitors

Inhibitors which strongly influence metabolic processes are also applied to distinguish between both uptake fractions. If the uptake into fraction B is an exchange-adsorption and the uptake into fraction C depends on metabolism, the rate of uptake into C will be decreased by metabolic inhibitors, whereas the rate of uptake into fraction B will not be influenced by these compounds.

In the first place the effect of HCN was studied, Arisz (1953, 1956) and Van Lookeren Campagne (1957) examined in detail the effect of HCN on the chloride uptake by *Vallisneria* leaves and their experiments showed among other things that the chloride uptake is strongly inhibited by HCN. It was to be expected that, directly or indirectly, also the cation uptake would be affected by HCN, Lundegårdh and Burström (1935). Fig. 9 shows three experiments in which the effect of different concentrations of HCN on the Rb uptake was investigated. The HCN stock-solution was made from a KCN solution by adjusting the acidity of the solution to pH 7 with the aid of HCl. The leaves in these experiments were not pretreated with HCN as it works almost instantaneously. This followed from other experiments

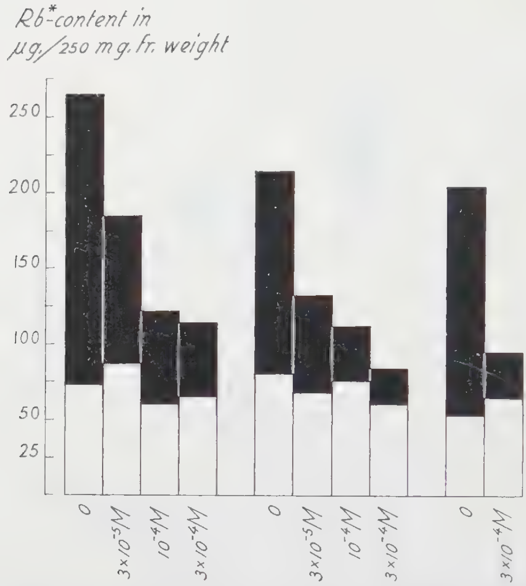

Fig. 9. Three experiments on Rb* uptake from a .008 M Rb*Cl solution for 60 minutes in the presence of 0, 3×10^{-5}, 10^{-4} and 3×10^{-4} M KCN. Black: Rb* fraction C; white: Rb* fraction B. Each column is the average value of two replications with the exception of the third experiment where each column represents the average value of 4 replications. KCl was added to the control to eliminate the effect of the K ions of the KCN solution on the Rb* fraction B.

in which the leaves were pretreated with HCN. Thus, it was sufficient to add the HCN only during the uptake period. By the HCN treatment the rate of uptake of the Rb-ions into fraction C is strongly inhibited, whereas fraction B is hardly influenced. The differences in size are not significant. Table 1 gives an impression of the variability of the determinations. The last two columns of Fig. 9 are derived from these values.

Sutcliffe (1954) and Lagerwerff (1956) could not find any effect of respiration-inhibitors on adsorption-exchange. On the other hand there are given in literature a few examples in which HCN affects the Apparent Free Space. (Bergquist, 1958; Lundegårdh, 1958). Fraction A and fraction B as defined in the foregoing are identical with the Apparent Free Space of Hope and Stevens (1952).

Bergquist (1958) showed that in the Brown-algae *Hormosira banksii* HCN increases the fraction A as well as the fraction B instead of decreasing it.

Lundegårdh (1958) however found that HCN inhibits the "Initial absorption" of Cl and K by wheat roots. He concludes from this that the "Initial absorption" is not completely non-metabolic. The "Initial absorption" is the first rapid uptake of ions by plant material when this is placed in a salt solution, so it can include, fraction A as well

TABLE 1

The effect of cyanide on the rate of uptake of Rb*.
Uptake period 60 minutes

External solution	Uptake of Rb in μg.					
	Fraction (B+C)	average	Fraction C	average	Fraction B	average
.008 M Rb*Cl solution + 3 × 10^{-4} M KCl.	210		158		52	
	205		153		52	
		205		153		52
	207		156		51	
	198		146		52	
.008 M Rb*Cl solution + 3 × 10^{-4} M KCN	106		29		77	
	91		28		63	
		94		31		63
	84		34		55	
	91		34		57	

as fraction B. Lundegårdh thinks that the "Initial absorption" is mainly adsorption-exchange, that would correspond with our fraction B.

The possibility exists, of course, that it is not allowed to compare a fraction B of one object with the fraction B of another, as the only common feature might be the exchangeability. Lundegårdh finds an adsorption-exchange of anions as well as for cations. Generally it is found however (KYLIN and HYLMÖ, 1957; LEGGETT and EPSTEIN, 1956), that the adsorption-exchange for anions is so small that it is hardly detectable. The discrepancy between the findings of Lundegårdh and other investigators can not be easily explained. However, whether the "Initial absorption" of Lundegårdh exists of a fraction A, a fraction B or a combination of both, the fact remains that HCN in his experiments, as opposed to mine with *Vallisneria* leaves, strongly affects the first rapid uptake of ions.

Just as HCN, the metabolic inhibitor Uranylnitrate strongly affected the rate of uptake of Rb-ions into fraction C and not the rate of uptake into fraction B (Fig. 10). Uranylnitrate was used because it was found by ARISZ (1958) that the chloride absorption was inhibited by Uranylnitrate. Uranylnitrate was added during the pretreatment as well as during the uptake period. In preliminary experiments it was found that it does not work immediately but only when it had been into contact with the leafstrips for several hours. The differences which are found in the reduction of the fraction B after treatment with the inhibitor are not significant.

Finally an attempt was made to influence the rate of absorption into fraction B with monoiodoacetamide, the inhibiting action of which on metabolic processes is a.o. based on combination with reactive SH groups. As with the other inhibitors the rate of uptake of Rb-ions into fraction C was nearly completely inhibited, but there

was no significant effect on the rate of uptake into fraction B (Fig. 11).

We may conclude from this chapter that whereas the formation of the fraction B depends upon physico-chemical features of the plant cell, the formation of the fraction C is dependent on its biochemical activity.

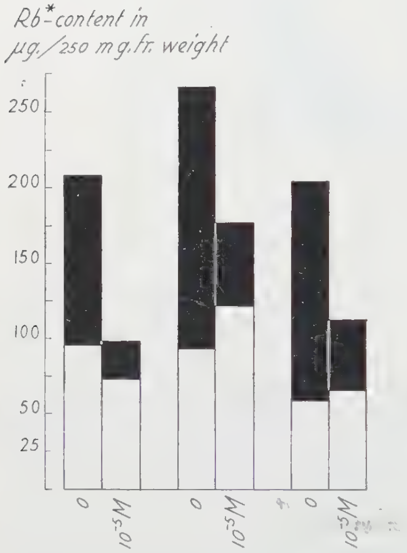

Fig. 10

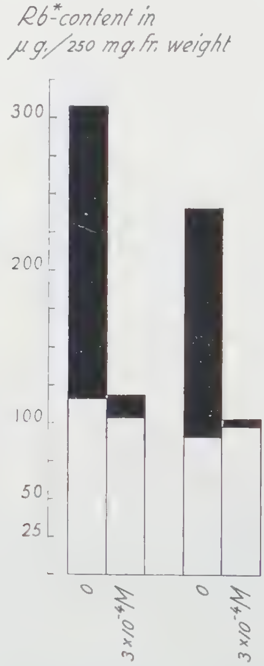

Fig. 11

Fig. 10. Three experiments on the effect of uranylnitrate on Rb* uptake from a .008 M Rb*Cl solution for 60 minutes. Black: Rb* fraction C; white: Rb* fraction B. Each column gives the average value of two replications.

Fig. 11. Rb* uptake from a .008 M Rb*Cl solution for 60 minutes after a two hours pretreatment in the dark in water or in a solution of 3×10^{-4} M monoiodoacetamide. Black: Rb* fraction C; white: Rb* fraction B. Each column represents the average value of two replications.

CHAPTER IV

THE RELATIONSHIP BETWEEN FRACTION B AND THE CONCENTRATION OF THE SURROUNDING SOLUTION

When leaves of *Vallisneria* are bathed in Rb-solutions the relationship between the fraction B and the external concentration proves to be nearly linear (Fig. 12).

When a fraction B of labelled rubidium ions has been established it is possible to remove the labelled ions by exchange for unlabelled rubidium. Fig. 13 shows that this exchange takes place in outer

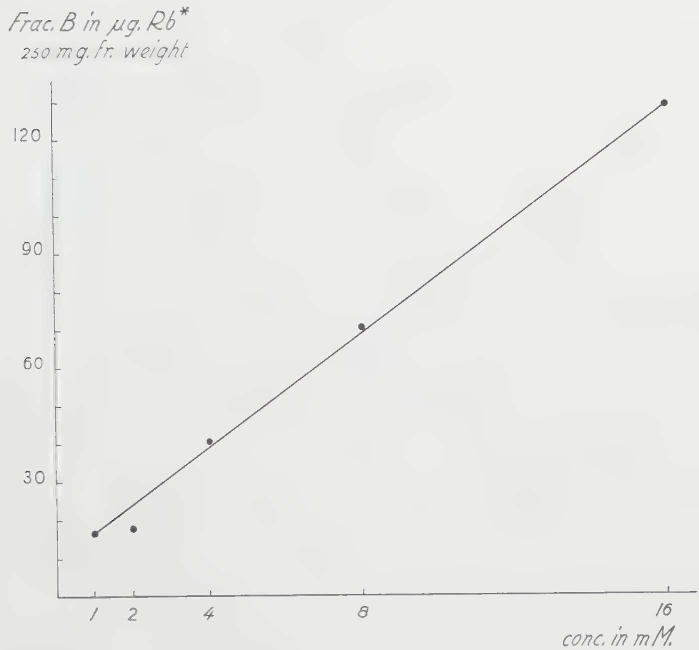

Fig. 12. The effect of the concentration of Rb*Cl on the size of the Rb* fraction B. Uptake for 60 minutes. Each point represents the average value of two replications.

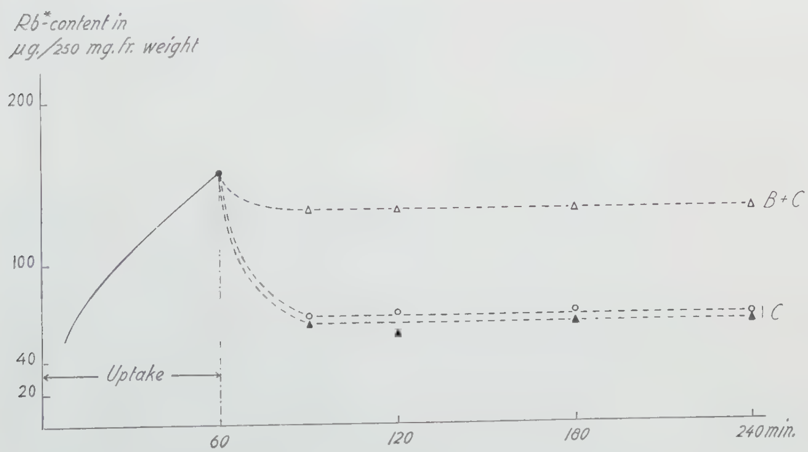

Fig. 13. Effect of the concentration of the unlabelled RbCl solution on the exchange of Rb* ions from fraction B. B and C refer to the fraction B and fraction C. ○——○ exchange in a .002 M RbCl solution; ▲——▲ exchange in a .010 M RbCl solution. Each point represents the average value of two replications.

solutions of .002 M and .010 M at the same rate. The most striking result is that the .002 M unlabelled sollution was as effective as the .01 M RbCl solutions with regard to removal of the exchangeable Rb-ions. Thus it seems that the establishment of a definite fraction B requires a higher external Rb concentration than the subsequent removal of this fraction by exchange.

Finally it was found that the exchange of a Rb fraction B is not very specific (Fig. 14). This figure shows that the Rb-ions of the

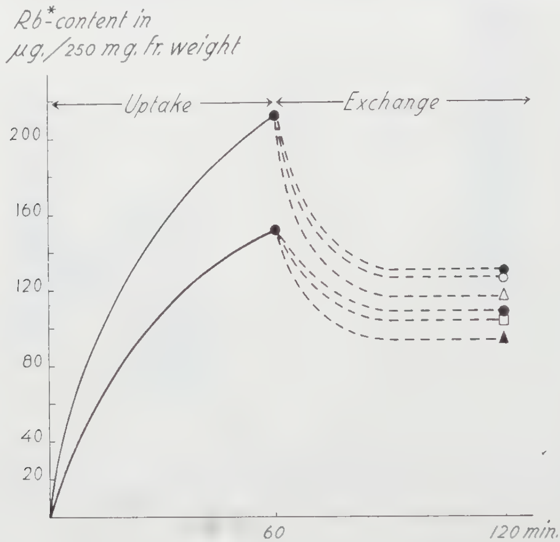

Fig. 14. Rb* uptake from a .008 M Rb*Cl solution for 60 minutes and subsequent exchange of the fraction B. ●——● exchange for RbCl .008 M; ●——□ exchange for KCl .008 M; ●——△ exchange for NaCl .008 M; ●——○ exchange for $MgCl_2$ .008 M; ●——△ exchange for $CaCl_2$ .008 M. Each point represents the average value of two replications.

fraction B can be exchanged for other cations than Rb as well, e.g. K, Na, Mg and Ca ions.

An explanation of these phenomena can be given if it is assumed that the exchangeable fraction B depends on a Donnan equilibrium.

A Donnan equilibrium exists when in a compartment ions are present which are unable to diffuse freely into the surrounding medium. If the compartment contains immobile anions at a concentration A, the concentration of the cations in this compartment should be the same to maintain electrical neutrality. For the sake of convenience all the cations may be supposed to be potassium. The concentration of cations will be equal to that of the anions, viz. A.

If KCl is applied outside of the compartment, potassium and chloride ions will diffuse into the space until the numbers of free ions which pass the boundary from left to right and vice versa are equal. These numbers depend on the products of concentrations of the ions. Thus:

$$\frac{[K] \text{ inside}}{[K] \text{ outside}} = \frac{[Cl] \text{ outside}}{[Cl] \text{ inside}}$$

When x K-ions and x Cl-ions have diffused through the membrane while A K-ions were already present for compensation of the immobile anions, then the equation holds that

$(A + x)\, x = C^2$ if C represents the concentration of the KCl in the external solution.

The result is that, K-ions are present in a higher concentration inside the compartment than outside of it.

When at this moment the KCl of the surrounding solution is replaced by other solutions, the potassium present in the Donnan compartment will be exchanged for other cations of the same sign. This process is not very specific.

If, besides monovalent cations, divalent and trivalent cations are present in the Donnan Compartment, the following equation holds:

$$\frac{[K^+] \text{ inside}}{[K^+] \text{ outside}} = \frac{\sqrt[2]{[Ca^{++}]} \text{ inside}}{\sqrt[2]{[Ca^{++}]} \text{ outside}} = \frac{\sqrt[3]{[La^{+++}]} \text{ inside}}{\sqrt[3]{[La^{+++}]} \text{ outside}}$$

This means that the distribution of the bi- and tri-valent ions is different from that of the monovalent ions. If the Donnan space contains immobile anions, the bi- and tri-valent cations will accumulate more in this space than the monovalent ions. Consequently the exchange of these ions for monovalent ions is more difficult than the reverse.

The ions in the Rb fraction B in *Vallisneria* leaves can be exchanged for other Rb-ions (Fig. 13). This is in agreement with the theory of Donnan equilibrium. If the theory is right there must be a compartment in the cells of *Vallisneria* leaves which contains immobile anions, a so called Donnan Space.

The charge of these immobile anions is neutralised by the Rb-ions and by other cations of fraction B. The fact that the cations of fraction B may be exchanged for various other cations is likewise in accordance with the theory of Donnan-equilibria. The uptake of ions into the fraction B is non-specific (Fig. 14). However, small differences in affinity for the monovalent cations may exist owing to different hydration of the cations (WILLIAMS and COLEMAN, 1949). Moreover Fig. 12 and 13 show that in introducing the Rb-fraction B into the cells, its size was dependent on the Rb concentration of the surrounding solution, whereas the exchange of the labelled Rb-ions for unlabelled Rb was independent of the outer concentration. This may be explained by assuming that at the start of the experiment part of the cations in the Donnan-space are not monovalent ions. We may safely assume that these "original" cations are partly calcium ions and, for a smaller part, magnesium-ions; as according to the Gibbs-Donnan distribution the Donnan-space has a higher affinity for calcium-ions than for the rubidium ions. Though the concentration of Ca outside of the Donnan-space will be extremely

small its concentration in the Donnan-space will remain relatively high as long as the ratio $\frac{\text{[Rb]-outside}}{\text{[Rb]-inside}}$ has a low value. This accounts for the strong dependence of the establishment of a fraction B on the Rb concentration of the outer solution.

If the Donnan-space contains Rb*-ions only, and these are exchanged for inactive rubidium ions (Rb°) equilibrium will be attained when

$$\frac{Rb^*}{Rb^\circ}\text{ inside} = \frac{Rb^*}{Rb^\circ}\text{ outside.}$$

Owing to the much larger volume of the outer solution as compared with that of the Donnan-space, the amount of unlabelled Rb will be much larger than the amount of labelled Rb even at low external concentration. Consequently virtually all of the Rb* of the Donnan-space will be exchanged for unlabelled rubidium of the medium. Only very low external Rb concentrations will be unable to remove the fraction B completely.

In order to test this theoretical explanation experiments were carried out in which the effect of a previous removal of the bivalent ions from the Donnan space was studied (Fig. 15). The open circles give the size of the Rb fraction B in leaves which had been treated with a .016 M solution of K_2SO_4 prior to the immersion in the solution of RbCl in order to remove most of the bivalent cations.

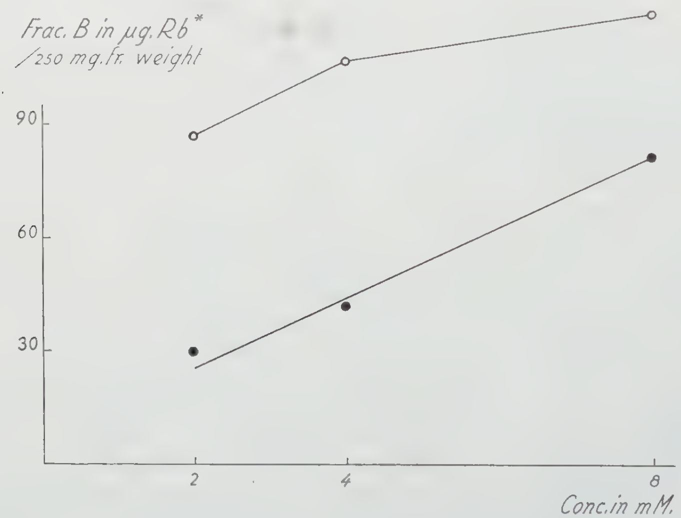

Fig. 15. The effect of a K_2SO_4 pretreatment on the size of fraction B. ●——● Rb* fraction B after uptake from Rb* solutions of different concentrations for 60 minutes. ○——○ Rb* fraction B after uptake from the same solutions by leaves that had been pretreated in a .016 M K_2SO_4 solution. Each point represents the average value of two replications.

The black dots give the size of the Rb fraction B of the control sets which had been bathed for a similar period in deionised water. Both sets were then allowed to absorb Rb from Rb*Cl solutions of various concentration for 60 minutes. The fraction B was determined in the usual way by exchange for unlabelled Rb. The fraction B of the control sets is almost proportional to the concentration of the labelled Rubidium solution.

The fraction B in the pretreated leaves is much higher than that of the leaves which had not been treated with K_2SO_4. Even in the lowest concentration of RbCl a large fraction B has been formed. Though in the higher concentrations of RbCl the fraction B is somewhat larger than in the lower concentrations, the linear proportionality has disappeared.

The small increase which was found may be ascribed to two causes. Possibly by the treatment of K_2SO_4, the bivalent ions originally present in the material, were not completely removed. Another possibility has been mentioned by Briggs, Hope and Pitman (1958). These authors assumed that the pH of the protoplasm increases with the concentration of the outer solution. This may cause an increase of the dissociation of the immobile anions in the Donnan-space, and, thus, an increase in number of the compensating counter ions, i.e. of the fraction B.

CHAPTER V

THE TRANSFER OF THE Rb FRACTION B TO THE FRACTION C AND ITS NATURE

1. The transfer of the Rb fraction B to the fraction C

In the previous chapters it was demonstrated that *Vallisneria* leaves possess an exchangeable cation fraction, the fraction B, just like numerous other plant tissues, e.g. roots, storage tissue, or algae. At the same time we tried to obtain an insight into the nature of this exchangeable cation fraction by studying the effect of some factors on this fraction. The second aim of this research was to study the relationship between the fraction B and the uptake of ions into the fraction C, in other words whether the fraction B is a link in the uptake of cations from the external solution into the fraction C.

First it was investigated if Rb-ions were transferred from fraction B to fraction C by a method after Epstein and Leggett (1954).

Vallisneria leafstrips were placed in a RbCl solution .008 M and after an uptake period of 60 minutes in some sets the fraction B and the fraction C were determined. The other sets were transferred to deionised water after rinsing off the adhering salt solution. At different intervals, ranging from 15 min. to 120 min., some of the sets were taken out of the water and used for the determination of the fraction C. The result is shown in Fig. 16. The Rb fraction C increases during the time that the leafstrips are in the deionised water.

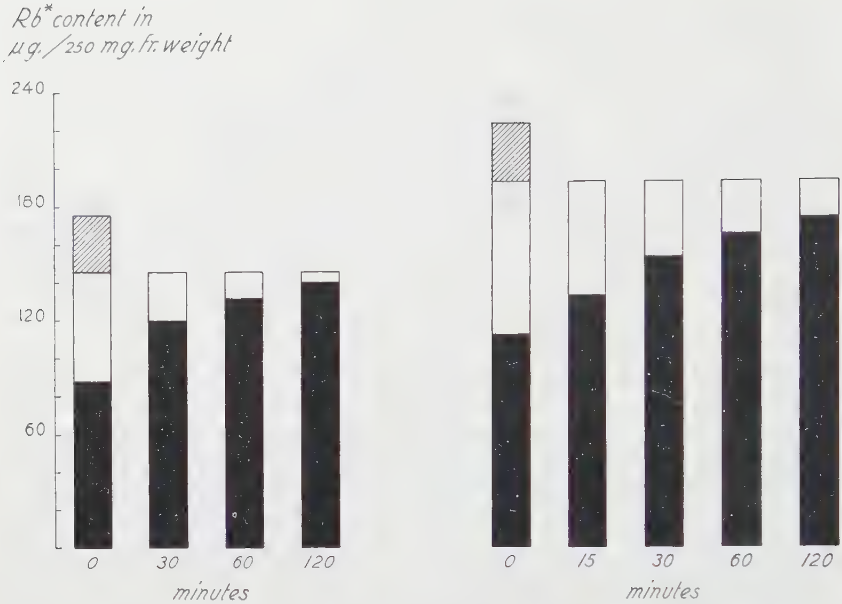

Fig. 16. Transfer of Rb* ions from the fraction B to fraction C in leaves kept in deionised water. Black: fraction C; white: fraction B; shaded: fraction A. Each point represents the average value of two replications.

Since the external solution has been removed this increase of the fraction C must be due to uptake of Rb-ions from fraction A, fraction B, or from both fractions.

However, the increase of the fraction C is such that it exceeds strongly the fraction A. Therefore, the increase cannot be ascribed to uptake of Rb-ions from the fraction A only. Before the leafstrips are transferred from the salt solution to deionised water, the greater part or perhaps the whole fraction A is washed out by dipping the leaves successively into four beakers with deionised water, to remove the adhering salt solution. This treatment takes 30 seconds. Then the leaves remain in deionised water for the time indicated in Fig. 16. It is assumed that the greater part of fraction A is removed by dipping the leaves in water during the first 30 seconds (Lundegårdh, 1949; Hope, 1953). What remains of fraction A diffuses into the surrounding water. These ions might be taken up into the fraction C. The volume of water in which the leafstrips remained was 1500 cc. The total fresh weight of the strips was at most 4 gram, containing a total fraction A of about 500 μ grams. This would give a concentration in the medium of 333 μ grams per 1000 cc i.e. 4 micromols of Rb-ions. Uptake from such a low concentration would be practically negligeable in the time of the experiment. Therefore the increase of the fraction C must be due to a transfer of Rb-ions from the fraction B to fraction C. This phenomenon is not restricted to *Vallisneria* leaves.

Epstein and Leggett (1954) already showed that an exchangeable Sr fraction of excised barley roots changed into an irreversible bound fraction. The rate of this change, however, was very slow. Also Brouwer (1959) showed that the exchangeable Rb fraction in excised pea roots could change into an unexchangeable fraction.

Finally G. de Leeuw 1957 (Unpublished results, Botanical Laboratory Groningen) found that the exchangeable Rb fraction in Beet-root disks (Sutcliffe, 1957) is transferred to fraction C.

2. The effect of monoiodoacetamide

In chapter III we saw that monoiodoacetamide strongly inhibits the rate of uptake of the Rb-ions from the external solution into the fraction C. We investigated now whether or not monoiodoacetamide could inhibit the transfer of Rb-ions from the fraction B to fraction C. The advantage of monoiodoacetamide is that it has no cations which could exchange for the Rb-ions of the fraction B.

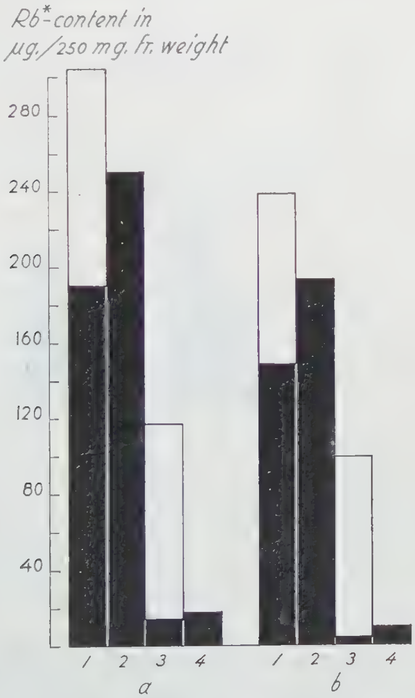

Fig. 17. Effect of monoiodoacetamide on uptake of Rb* ions into the fraction C and on the transfer of Rb* from fraction B to fraction C. a_1 and b_1: Rb* uptake from a .01 M Rb*Cl solution for one hour. Black: Rb* fraction C; white: Rb* fraction B. a_2 and b_2: Rb* fraction C in leaves treated as in a_1 and b_1 and than transferred to deionised water for two hours. a_3 and b_3: Rb* uptake from a .01 M Rb*Cl solution for one hour after a two hours pretreatment with monoiodoacetamide 3×10^{-4} M. a_4 and b_4: Rb* fraction C in leaves treated as in a_3 and b_3 and then transferred to deionised water for two hours. Each column represents the average value of two replications.

During studying the transfer of the Rb-ions of the fraction B to fraction C, the leafstrips must be placed in deionised water to be sure that only ions of the fraction B and not from the medium could reach the fraction C. It appeared that pretreatment of the *Vallisneria* leafstrips with monoiodoacetamide is sufficient to inhibit the subsequent uptake of cations, so that there was no need to supply the monoiodoacetamide to the water in which the leaves remained during the study of the transfer of fraction B to fraction C. However, to obtain an almost complete inhibition of the uptake of cations into the fraction C it was necessary to supply the monoiodoacetamide at least 2 hours before starting the experiment. The results are shown in Fig. 17. As was found in chapter III the size of the fraction B is not affected by the monoiodoacetamide. The present result shows that the transfer of Rb-ions from fraction B to fraction C is strongly inhibited by the monoiodoacetamide.

3. The effect of temperature

The Q_{10} of the transfer of Rb-ions from B to C may give information about the nature of this process (Chapter III). For this reason the effect of temperature on the rate of this transfer was studied. The result is given in Fig. 18.

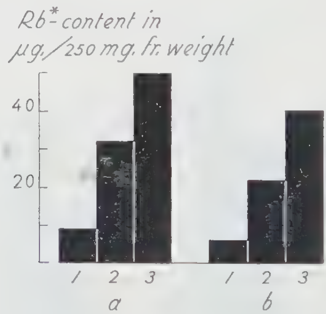

Fig. 18. Effect of temperature on the transfer of Rb* from fraction B to fraction C. a_1 and b_1: Rb* fraction C after uptake from a .01 M Rb*Cl solution in the dark for one hour. a_2 and b_2: Rb* fraction C after a subsequent 30 minutes stay in deionised water in the light at a temperature of 18° C. a_3 and b_3: Rb* fraction C after a similar treatment but at a temperature of 27° C. Each column represents the average value of 4 replications.

To study the effect of temperature on the transfer of Rb-ions of fraction B to fraction C with sufficient accuracy, it was desirable to keep the fraction C during the uptake period as small as possible. In preliminary experiments it was found that the rate of Rb uptake in the fraction C is much lower in the dark than in the light, but that light does not affect the size of the fraction B. For this reason leaf strips were placed in a RbCl solution in the dark for one hour. Moreover, the temperature during the uptake of Rb was rather low, viz. 20° C. After uptake, the strips were rinsed with water and then placed in deionised water at different temperatures.

To obtain a higher degree of accuracy the number of the sets taken for one determination was 6 or 8. During the transfer of Rb from fraction B to fraction C, that is, during the time that the leafstrips were in deionised water, the leafstrips were illuminated.

In Fig. 18a we see that the average uptake of Rb-ions into the fraction C during the uptake period was very low indeed, viz. 9 μg. The transfer from B to C during 30 minutes in water at a temperature of 18° C is 23 μg and at a temperature of 27° C 41 μg. The Q_{10} is 2,1. In Fig. 18b the Q_{10} is 2,15. We may conclude, therefore, that temperature affects the transfer of Rb from fraction B to fraction C strongly, though the temperature quotient is somewhat lower than could be expected for an active transport process. A possible explanation for this discrepancy will be given in chapter VI.

The main conclusion drawn from this chapter is, that the transfer of Rb-ions from fraction B to fraction C as well as the uptake of Rb-ions from the external solution into fraction C depend on metabolism.

CHAPTER VI

THE RATE OF THE TRANSFER OF Rb FROM THE EXCHANGEABLE FRACTION B TO THE IRREVERSIBLE FRACTION C

1. Statement of the problem

Fig. 19, which gives the transfer of Rb from fraction B to fraction C versus time, shows that the rate of this transfer decreases with time.

In the beginning the transfer proceeds rapidly and is of the same magnitude as the previous rate of uptake of Rb-ions into the fraction C from the salt solution, but after half an hour there is a sharp decrease

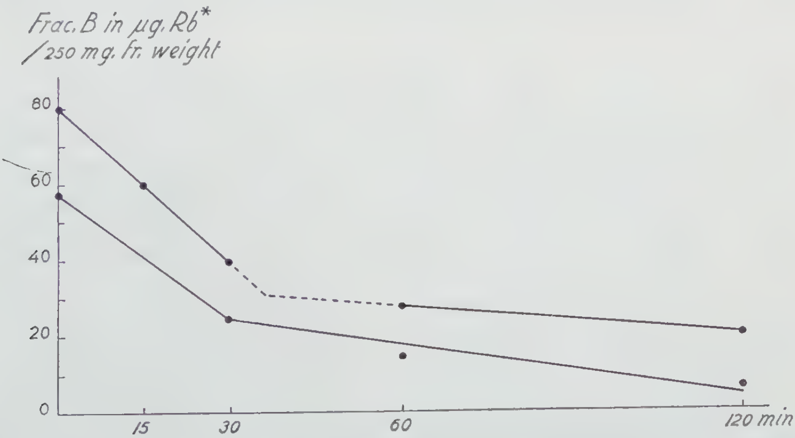

Fig. 19. The decrease of the Rb* fraction B during the time that the leaves are in deionised water. Each point represents the average value of two replications.

in the rate of transfer and even after two hours the fraction B has not been transferred completely to fraction C. This decrease of the rate of uptake occurs only when the leaves are in water. If leaves are bathed in a salt solution the fraction C increases at a constant rate. This will be due to the fact, that when the leaves are in a salt solution the quantity of Rb-ions available for uptake remains almost constant, whereas, when the leaves are in water the size of the fraction B decreases during the transfer to C. When the rate of the transfer is directly proportional to the size of the fraction B, the line which represents the decrease of the Rb fraction B must be a straight line if plotted semilogarithmically (Fig. 20). However, the decrease is

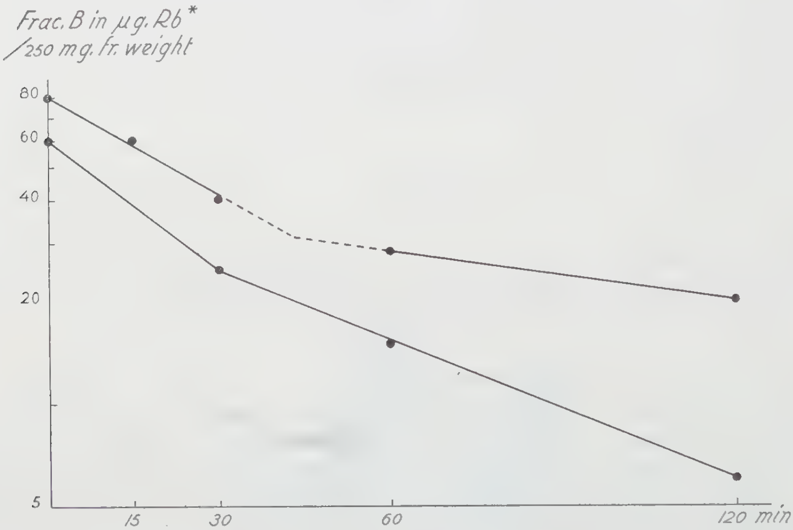

Fig. 20. The results of the experiments, shown in figure 19, plotted semilogarithmically.

more rapid then could be expected when transfer is proportional to the size of fraction B, thus the decrease in the quantity of Rb-ions of the fraction B is not the only factor which causes the decrease in the rate of transfer. Another important difference between uptake from a salt solution and the transfer from B to C while the leaves are in water is the absence of the accompanying anions (Cl or SO_4).

That no ions are present during the transfer (of the Rb from fraction B to fraction C) is clear from the fact that the leaves are transferred from a salt solution to deionised water after several subsequent rinsings in deionised water. During the rinsings, the adhering solution is removed and in doing so also the greater part of the anion and cation fraction A is washed out. For anions a fraction B might be present but it could not be detected in measurable amounts and can, therefore, be neglected (Fig. 5).

The question arises, whether or not the absence of the accom-

panying anion slows down the speed of the transfer of the Rb from fraction B to fraction C.

2. The effect of anions on the rate of the transfer

It is an axiomatic law that the total electrical charge of ions inside plant cells must be equal to zero.

This implicates that either there is an equal uptake of anions and cations by the plant or an exchange of ions which are already present in the plant for ions of equal charge which are taken up. The former possibility was suggested by Hurd and Sutcliffe (1957) and by Hurd (1958) who found that the total uptake of bicarbonate and chloride ions in beetroot disks was equal to the potassium uptake. The second possibility may imply that an excess uptake of added cations is balanced by hydrogen ions which are given off to the external solution in barter for these cations (Jacobson and Ordin, 1954). If accumulation of a cation is only possible when at the same time this cation is joined by an anion, a deficit of accompanying anions might limit the transfer of the Rb from fraction B to fraction C. The number of cations and anions taken up by identical leaves was investigated as follows. A batch of leaves was divided into two equal sets.

One set was bathed in a solution of potassium chloride, the chloride of which contained Cl–36. The other set was bathed in rubidium chloride, the rubidium of which contained Rb–86. The uptake of Cl–36 from the KCl, and the uptake of the Rb–86 from the RbCl were determined as counts per minute, the uptake of chloride from RbCl by Volhard's method. The results are given in table 2 and Fig. 21, calculated as μ equivalents per 250 milligrams

Table 2

Uptake of Rb* from a .008 M Rb*Cl solution and of Cl* from a .008 M KCl* solution

Uptake period	External solution							
	Rb*Cl				KCl*			
	isotope technique Rb* in μeq		Volhard Cl in μeq		isotope technique Cl* in μeq		Volhard Cl in μeq	
60 min.	1,86	1,76			1,41	1,50	1,01	1,21
	1,66				1,58		1,41	
120 min.	3,45	3,30	3,25	3,05	2,79	2,92	2,90	3,10
	3,15		2,85		3,05		3,30	
180 min.	4,71	4,60	4,54	4,24	4,54	4,44	4,62	4,72
	4,50		3,94		4,34		4,83	

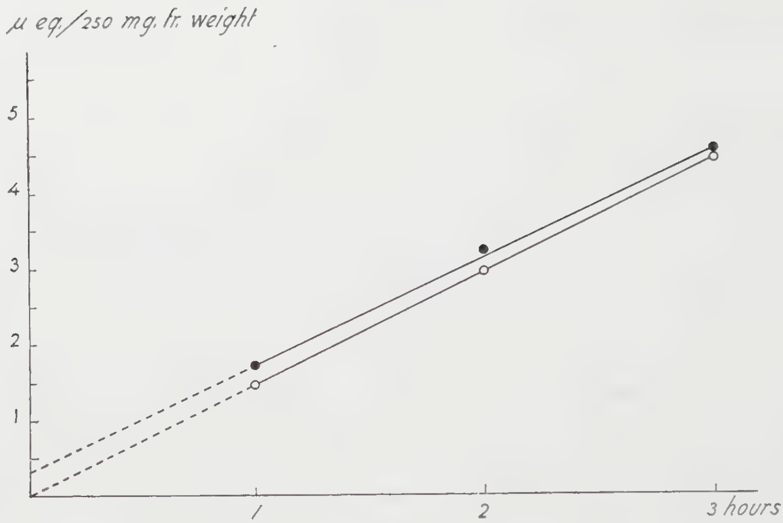

Fig. 21. Course of irreversible rubidium and chloride absorption. ●——● Rb* fraction C after uptake from a .008 MRb*Cl solution; ○——○ Cl* fraction C after uptake from a .008 MKCl* solution. Each point represents the average value of two replications.

fresh weight. Chloride and rubidium ions are taken up in practically equivalent amounts. The uptake of rubidium is slightly higher than that of chloride. If this difference is significant it might be due to the fact that Cl was taken up from a KCl solution. For technical reasons the uptake of labelled rubidium and labelled chloride was not determined from a solution of Rb–86 Cl–36. Another cause for the difference may be that the rubidium fraction B had not completely been removed from the material before C was determined.

A third possibility is that the small excess of Rb-uptake over chloride-uptake was due to the presence of bicarbonate ions. A similar set of experiments was carried out to determine the uptake of sulphate and of rubidium by identical leaves.

Fig. 22 shows that the amounts taken up are not equivalent, the rubidium uptake strongly exceeding the uptake of sulphate-ions. To maintain the theory that the uptake of an anion is coupled with the uptake of a cation, one must assume that here bicarbonate ions join the Rb-ions to compensate for the excess cation uptake and that this can be maintained at a constant speed for several hours. When studying the transfer of Rubidium ions from B to C the presence of bicarbonate ions in the deionised water cannot be prevented.

The bicarbonate concentration may be decreased by lowering the pH of the water, but this procedure would cause the exchange of the Rb-ions from the B fraction against hydrogen ions. For that reason a pH at which a certain concentration of bicarbonate ions exists can not be avoided.

Therefore, the decrease of the rate of transfer of the Rb fraction B to C, which was described in chapter v, might be due to a depletion of the bicarbonate ions. This hypothesis is not supported by the results of Fig. 22, which shows that, under comparable conditions, the excess uptake of Rb over SO_4 may proceed for several hours. This shows, that if this excess uptake is due to bicarbonate ions the

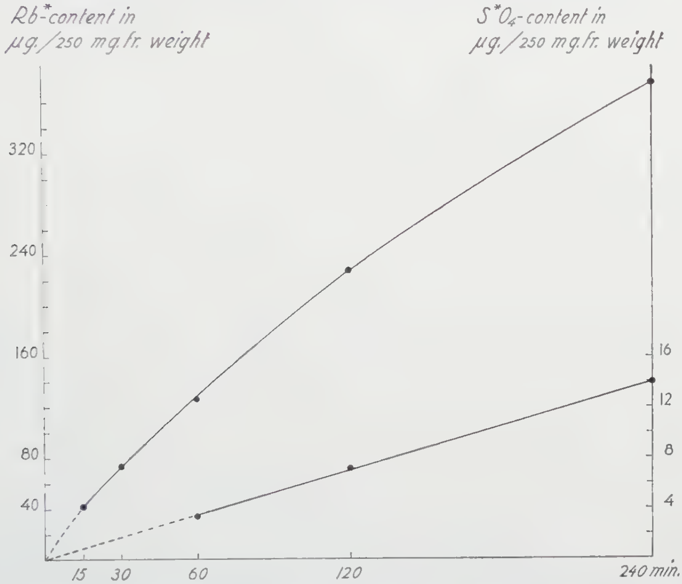

Fig. 22. Course of irreversible rubidium and sulphate absorption. Upper curve Rb* fraction C after uptake from a .004 M $Rb^*{}_2SO_4$ solution. Lower curve SO_4 fraction C after uptake from a .004 M $K_2SO^*{}_4$ solution. Each point represents the average value of two replications.

supply of bicarbonate ions allowing the excess uptake is not exhausted within that period.

In the experiments on the transfer of the Rb-ions from B to C, the rate drops sharply after half an hour. It seems unlikely that in the latter experiments the supply of bicarbonate ions would diminish at a different rate from that in the experiment of Fig. 22 and thus, that the rapid decrease of the rate of transfer from B to C is caused by exhaustion of the stock of bicarbonate or of other anions.

It may be supposed that the anions required for the transfer of Rb-ions from B to C, do not form a limited stock, but that they are generated by metabolism. Then, the decrease in rate of the transfer of Rb-ions from B to C might be due to the low rate at which these anions are formed. The following experience makes this supposition rather improbable. After two hours in water the rate of transfer of Rb from B to C has decreased considerably. When at this moment the leafstrips are placed for a second time in a solution of RbCl for one hour, a Rb-fraction B will originate for the second time.

If the rate at which the anions are formed determines the rate of transfer of Rb-ions from B to C, it must be expected that, when the leaves are put in water again, the second B fraction is transferred to C very slowly. However, the opposite is true: the transfer proceeds again at a high initial rate (Fig. 23).

From these data it appears highly unprobable that a decrease in the quantity of the available anions causes the decrease in the rate

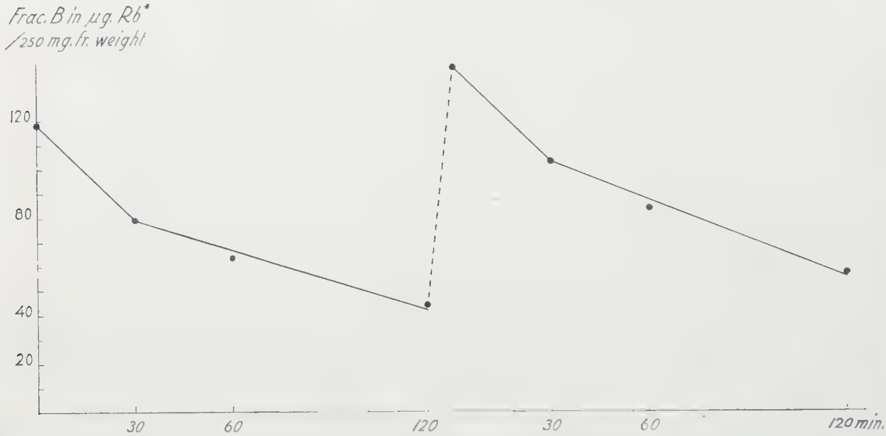

Fig. 23. Leafstrips previously bathed in a .010 M Rb*Cl solution. The left part of the figure shows the decrease of the fraction B after transfer of the leafstrips to deionised water. After 120 minutes in deionised water the leafstrips were again bathed in a .010 M Rb*Cl solution. The right part of the figure shows that, after transfer to deionised water, the fraction B decreases at the same rate as the first time. Each point represents the average value of two replications.

of the transfer. One could object to this that the production of these anions is only possible, when the leaves are bathed in a salt solution and not when the leaves are bathed in deionised water owing to the salt respiration which will doubtlessly rise and may eventually influence the forming of the anions. But it is known from (LUNDEGÅRDH, 1949) that the anion respiration decreases in rate only slowly when plant tissues are transferred from a saltsolution to distilled water.

A period of 120 minutes in deionised water is too short to decrease the rate of the anion respiration to a considerable extent. Summarizing we may conclude that, if anions are required for the transfer of Rb-ions from B to C, their quantity will not become the limiting factor for the rate of this transfer.

3. THE EFFECT OF THE CONCENTRATION OF Rb IN THE FRACTION B ON THE RATE OF TRANSFER FROM B TO C

From the foregoing it is highly probably that the quantity of anions does not determine the rate of transfer of Rb-ions from B to C. For that reason we must assume that it is the number of Rb-ions present

in B that determines this rate. In the experiment of table 3 fractions B of different size were obtained by placing the leaves in a .004 M RbCl or in a .008 M RbCl solution.

TABLE 3

Transfer of Rb*ions from fraction B to fraction C during the first half hour

	Fraction B in μg initial	Fraction B in μg after 30 min. in water	Transfer in μg
Expt 1	40	17	23
	66	25	41
Expt 2	58	34	24
	94	46	48

The rate of transfer is about linear to the initial size of the fraction B, during the first 30 minutes.

The results of table 4 show that after the first 30 minutes the rate of transfer from the exchangeable fraction to fraction C is always smaller than the initial rate in comparable leaves, even if the concentrations of the exchangeable fraction are equal. In the experiment of table 4 to one part from a batch of leaves of *Vallisneria* was given a large fraction B, (B_1) to the other part a much smaller fraction (B_2). This is attained by placing the first part for one hour in a .008 M RbCl solution and the second part for the same time in a .004 M solution. Subsequently the leaves are placed in water and

TABLE 4

Rate of transfer of Rb* ions from fraction B to fraction C.
In each experiment half of the leaves was given a large Rb* fraction B, the other half a much smaller fraction. The leaves were transferred to water and fraction B was determined

Time after transfer of the leaves to water	0′	30′	60′	120′
Expt 1. Large fraction B_1(μg) . . .	78	66	55	47
average $-\triangle B_1$/30 min. . .		12	11	4
small fraction B_2(μg) . . .	48	40		
$-\triangle B_2$/30 min.		8		
Expt 2. Large fraction B_1(μg) . . .	66	50	45	44
average $-\triangle B_1$/30 min. . .		16	5	0,5
small fraction B_2(μg) . . .	41	31		
$-\triangle B_2$/30 min.		10		
Expt 3. Large fraction B_1(μg) . . .	75	55	51	47
average $-\triangle B_1$/30 min. . .		20	4	2
small fraction B_2(μg) . . .	44	31		
$-\triangle B_2$/30 min.		13		

the Rb in fraction C is determined at intervals. Since in water the Rb of fraction B cannot decrease by exchange, the increase of Rb ions in fraction C is equal to the number of ions transferred from B to C ($-\triangle B$). The results are shown in table 4. During the first 30 minutes the $-\triangle B$ is about linear to the size of fraction B at the start of the experiment.

The ratio's of the large and the small fraction $\frac{B_1}{B_2}$ at the start of the experiment are:

$$\frac{78}{48} = 1{,}6 \text{ (Expt 1)}, \frac{66}{41} = 1{,}6 \text{ (Expt 2)}, \frac{75}{44} = 1{,}7 \text{ (Expt 3)}$$

The corresponding ratio's of the transfer from B to C $\frac{\triangle B_1}{\triangle B_2}$ in the first 30 min are:

$$\frac{12}{8} = 1{,}5 \text{ (Expt 1)}, \frac{16}{10} = 1{,}6 \text{ (Expt 2) and } \frac{20}{13} = 1{,}5 \text{ (Expt 3)}.$$

After 60 minutes the large fraction B_1, has decreased to a value only slightly higher than that of the small fraction B_2 at the start of the experiment. If the proportionality between the number of ions in B and the transfer to C had been maintained, in the next 30 minutes, the value of $-\triangle B_1$ should be about equal to that of $-\triangle B_2$ in the first thirty minutes.

However, these values are widely different, viz 4 and 8, 0,5 and 10, 2 and 13. This proves that the Rb ions which are still in B after 60 minutes are transferred at a much smaller rate than Rb-ions at the start of the experiment.

An explanation which may be given for this phenomenon is based on the hypothesis that various types of cells show a difference in the rate of accumulation of ions. That part of the Rb fraction B which is located in the cell-walls of strongly accumulating cells, will be transferred rapidly to C, whereas the part which is located in the cell-walls of the slowly accumulating cells will be transferred very slowly. That the Rb fraction B is located in the cell-walls of all the cells of a leaf is supported by the fact that exchange of Rb in *Potamogeton* can occur readily across the whole leaf (Helder, unpublished results).

It was shown in chapter v that the Q_{10} of the transfer of Rb-ions from fraction B to fraction C was lower than could be expected for an active transport process. In this chapter it has been shown that the rate of the transfer depends on the size of the Rb fraction B. This fact makes it understandable why the Q_{10} of the tranfer was relatively low. At the high temperature the concentration of the ions of fraction B will decrease at a greater rate than at the low temperature. This decrease will counteract the temperature effect.

CHAPTER VII

THE ROLE OF THE EXCHANGEABLE FRACTION B IN THE IRREVERSIBLE ABSORPTION OF IONS FROM THE EXTERNAL SOLUTION TO C

1. The effect of the size of the fraction B on the rate of increase of the fraction C for Rubidium ions

As has been discussed in chapter VI, the initial rate at which the Rb-ions are transferred from fraction B to fraction C while the leaves are in water, is of the same magnitude as the rate of uptake of Rb-ions into the fraction C when the leaves are bathed in a salt solution. This fact suggests that uptake into the fraction B is a step in the uptake of ions from the medium into the fraction C. But the possibility remains that, normally, ions are transferred directly from A to C. These ions will compete with ions from B for the accumulating mechanism, and, by this, the transfer from B to C may be more or less blocked. The conditions under which this transfer was studied might well have been exceptional, because rubidium ions were absent from the external solution and fraction A.

If one studies Fig. 24, it is striking that the rate of rubidium uptake into the fraction C is nearly independent of the Rb concentration of the external solution, at least within the concentration range which was investigated. Consequently, it is also independent of the size of the fraction B, as this fraction shows an almost linear relationship with the concentration of the external solution. It may be concluded, that, if fraction C is formed by way of the fraction B, a small fraction B is sufficient for a high rate of transfer from B to C.

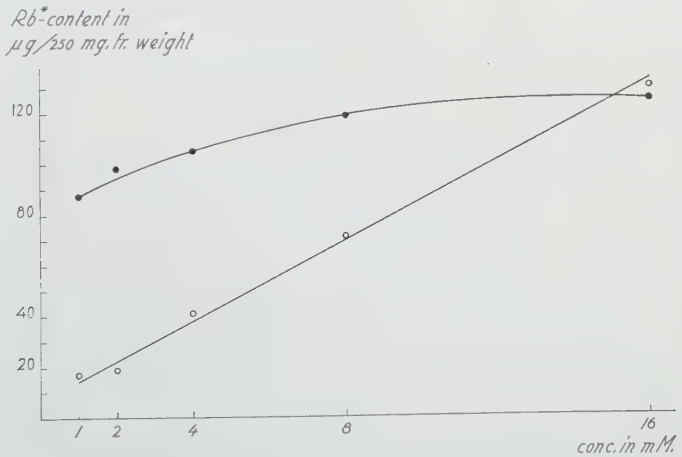

Fig. 24. The relationship between external concentration and the size of fraction B and C for rubidium ions. ●——● the rate of uptake into the fraction C; ○——○ the size of the Rb* fraction B. Each point represents the average value of two replications.

On the contrary, when no rubidium ions are present in the external solution, the initial rate of the transfer of the rubidium from fraction B to fraction C does depend on the size of the fraction B even when this size is great (Chapter VI).

Thus, in contrast to the previous finding this result suggests that a high rate of the transfer is not possible when fraction B is small. This contradiction indicates that fraction B is not intermediate in the uptake of Rb-ions from the external solution into the fraction C.

2. The transfer of Ca-ions from fraction B to fraction C

Other indications that uptake of Rb-ions into the fraction B is not a step in the uptake of cations from the external solution into fraction C, follow from experiments with $CaCl_2$. The method of studying Ca–45 uptake has been described in chapter I in the section on methods. Table 5 shows that, just as with Rb-ions, the Ca-ions

Table 5

Uptake of Ca* from a Ca^*Cl_2 solution

Uptake period	Uptake of Ca* in μg				
	Fraction C		Fraction B+C		Fraction B
1 hour	2,4 2,6	2,5	71,5 70,0	70,8	68,3
4 hours	8,6 8,2	8,4	85,8 89,1	87,5	79,1
8 hours	15,4 16,5	16,0	96,6 92,7	94,7	78,7
24 hours	31,2 31,9	31,6	117,9 114,7	116,3	84,7

absorbed could be separated into fraction B and C. The fraction B appears to be smaller after an uptake period of one hour than after uptake periods of 4, 8 and 24 hours. The differences are probably significant and it must be concluded that, as opposed to the fraction B for rubidium, the Ca-fraction B is not saturated within one hour. This also implies that 60 minutes is not long enough a period for a complete exchange of the labelled Ca of fraction B for unlabelled Ca-ions from the medium. This is in agreement with the findings of Briggs, Hope and Pitman (1958) who found the same for beetroot disks. The time of exchange in this experiment was therefore 4 hours. The amount of Ca-ions of the fraction C increases with time, but the rate of uptake into fraction C decreases. Furthermore it can be seen that the rate of calcium uptake is very low as compared to the Rb uptake. Next the transfer of Ca from fraction B to fraction C was examined (Fig. 25).

The white column gives the size of the Ca fraction C after an

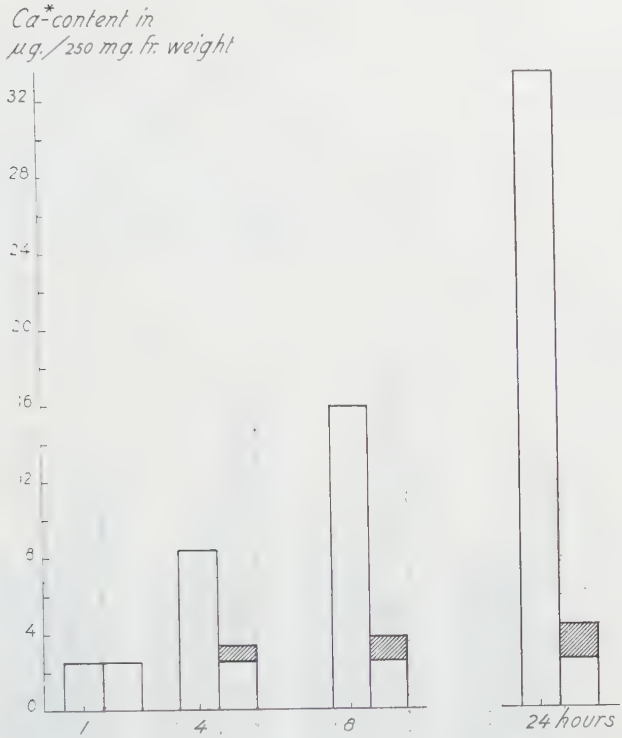

Fig. 25. The course of irreversible calcium absorption and the transfer of calcium from fraction B to fraction C. The left white columns represent the Ca fraction C after uptake from a .002 M Ca*Cl_2 solution for various periods. The right white column represent the Ca* fraction C after uptake from a .002 M Ca*Cl_2 solution for one hour and after that in water for the time indicated at the base of the adjoining columns. The shaded columns represent the course of the transfer of Ca*ions from the fraction B to fraction C. Each column represents the average value of two replications.

uptake period of one hour and the shaded column the transfer of Ca from fraction B to fraction C with time. The transfer of Ca-ions from the fraction B to fraction C, though significant, is extremely small and in no proportion to the rate of the uptake of Ca-ions from the external salt solution into fraction C.

That a transfer of Ca-ions could hardly be detected cannot be due to a small size of the fraction B for this is large as compared with the size of the fraction B for rubidium as far as the number of ions is concerned. The main conclusion drawn from this experiment is that it is possible to obtain a fraction C for Calcium by absorption directly from the external solution instead of via the fraction B. This means that the fraction B is not a step in the uptake of Ca-ions into the fraction C, and this supports the hypothesis that also for rubidium the fraction B is not a necessary step in the uptake of Rb-ions into the fraction C.

It is not certain, however, if the uptake of the Rb into fraction C must be attributed to the same mechanism responsible for the uptake of the Ca into fraction C. Yet it could be proved that uptake of Ca into the fraction C is a metabolic process, for it could be inhibited by cyanide. Moreover this is the same inhibitor that inhibited the Rb uptake into the fraction C. The result of these experiments are shown in Fig. 26. This figure shows that $Ca(CN)_2$ inhibits the rate of the uptake of Ca-ions into the fraction C. On the other hand, table 6 shows that the size of the fraction B is not affected by cyanide.

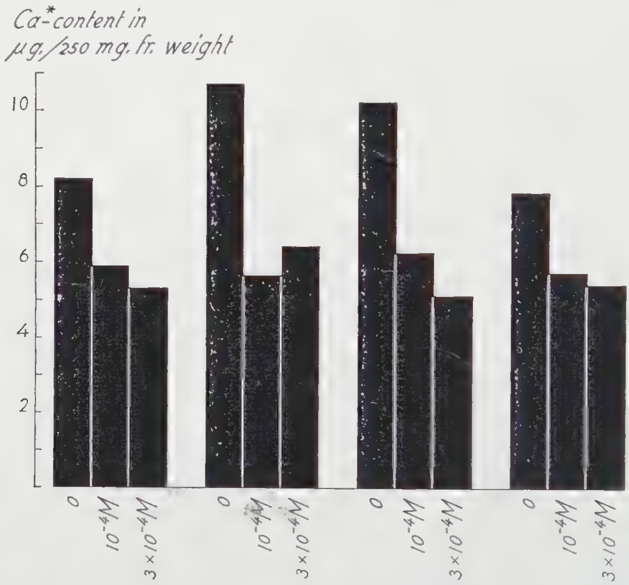

Fig. 26. The effect of 10^{-4} and 3×10^{-4} M $Ca(CN)_2$ on the rate of uptake of Ca ions into the fraction C in four different experiments. Uptake from a .002 M Ca^*Cl_2 solution for 4 hours. Each column represents the average value of two replications.

TABLE 6

The effect of $Ca(CN)_2$ on the size of the Ca* fraction B
1, 2, 3 and 4 give the average Ca fraction B in µg/250 mg fresh weight in separate experiments. Uptake period 4 hours.

External solution	Experiment nr.				
	1	2	3	4	Average
Ca^*Cl_2.002 M	96	94	73	74	84
Ca^*Cl_2.002 M + 10^{-4} M $Ca(CN)_2$	98	94	74	75	85
Ca^*Cl_2.002 M + 3×10^{-4} M $Ca(CN)_2$	90	75	84	78	82

3. The effect of calcium on the uptake of rubidium

Further evidence for the hypothesis that uptake into fraction B is not necessary for uptake of ions into fraction C, has been obtained by studying the uptake of Rb-ions into the fraction C when both Ca-ions and Rb-ions were present in the external solution. It was discussed that the "accumulation" of Ca-ions in the fraction B exceeds strongly the monovalent Rb-ions when both are present in the external solution. So it is possible to fill the Donnan Space with Ca-ions instead of with Rb-ions.

If the fraction B is a necessary step in the uptake of ions into the fraction C, no active uptake of Rb-ions would have occurred under these conditions. Fig. 27 shows the result of this experiment. The

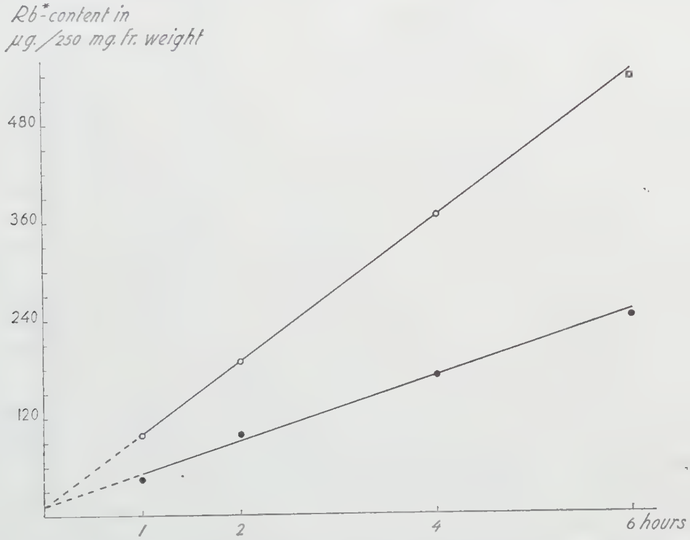

Fig. 27. The influence of calcium on the irreversible rubidium absorption. ○——○ Rb* fraction C after uptake from a .001 M Rb*Cl solution for various periods. ●——● Rb* fraction C after uptake from a .001 M Rb*Cl + .010 M $CaCl_2$ solution. Each point represents the average value of two replications.

concentration in the external solution of Rb was .001 M. Fig. 24 shows that at that concentration the fraction B is very small, namely about 15 μg R b/250 mg fresh weight. The calcium concentration was 10 mM. It was discussed in chapter IV that this will decrease the Rb-ions in B far below 15 μg/250 mg fresh weight. In fact, the amount was so small that it was impossible to determine its actual value. Fig. 27 shows that though Ca decreases the rate of Rb uptake by about 50 %, the rate is still considerable.

Since the rate of transfer from B to C in the absence of ions in the external solution depends on the size of fraction B (table 3) hardly any transfer should have been obtained. From the relatively high uptake of Rb in the presence of calcium it must be concluded that the ions were transferred directly from A to C.

CHAPTER VIII

THE LOCATION OF THE FRACTION B IN THE PLANT CELL

One of the first authors who demonstrated the presence of an exchangeable cation fraction within plant cells and expressed an opinion about its location was DEVEAUX (1896). Deveaux postulated that the exchangeable cation fraction is mainly in the cell-wall where the cations are "electrostatically bound" to the pectins. In this, Deveaux has been supported by the results of various workers MATTSON (1949), KELLER und DEUEL (1957), DAINTY and HOPE (1960), JANSSENS *et al* (1959), MERTZ and LEVITT (1961) etc.

Mattson pointed out that, after staining the roots of different cereals with methylen-blue, this dye could microscopically be shown to be present in the cell-wall and that the same structures were stained when a colour reaction on pectin was carried out. Moreover, this dye was exchangeable for Ca-ions. KELLER und DEUEL (1957) showed that 70–90 % of the cation exchange capacity of the roots of various plants as corn, barley, tobacco and tomato's must be ascribed to the free carboxyl groups of the pectins which are present in the cell-wall. JANSSENS *et al.* (1960) obtained almost the same results for Avea-coleoptiles. Also DAINTY and HOPE (1960) who reconsidered the opinion of MC ROBBIE and DAINTY (1958) that the exchangeable cation fraction has to be localised in the protoplasm of the plant-cell, found that nearly all the easily exchangeable cations in the internodal cells of *Chara australis* are present in the cell-wall.

Other workers e.g. LUNDEGÅRDH (1941), BUTLER (1953), HOPE and PITMAN (1958) and SUTCLIFFE (1954) have opposed the idea of a location in the cell-wall. They claim that the exchangeable cation fraction is chiefly located in the cytoplasm.

Lundegårdh based his opinion mainly on his idea that the Donnan potential between roots and surrounding solution, which originates from the unequal distribution of the cations between the Donnan phase and the surrounding solution, does not exist between cell-wall and surrounding solution but between protoplasm and surrounding solution. As an argument he advances that destruction of the cytoplasmic membrane by heating or by a treatment with alcohol completely alters the picture of the potential change which is induced by dilute acids. While in living cells a change in the concentration of the hydrogen ions of the surrounding solution causes a change in this potential within 0,75 seconds, this change arises only very slowly in cells killed by heating or by alcohol. This, however, does not rule out a location of the Donnan phase in the cell-wall, because pectins are also influenced by heating, (HEINTZE, 1961).

BUTLER (1953) located the fraction A (W.F.S.) partly in the cytoplasm, because a free space of 20 % to 25 % was too high to locate it completely in the cell-wall. This means that ions can pass the plasmalemma by diffusion. If this is true, part of the cation adsorption-exchange might also be located in the cytoplasm, because

the cytoplasm surely contains negatively charged immobile anions.

LEVITT (1959), however, suggested that the W.F.S. of 20–25 % found by Butler is not a pure W.F.S. but partly due to a microscopically thin film of liquid on the surface of the roots.

SUTCLIFFE (1954) found that the exchangeable fraction in beetroot disks decreases when they are rinsed in distilled water at 5° C.

Since, according to Sutcliffe, it was not very likely that, during rinsing, the composition of the cell-wall changes, the effect must be ascribed to the cytoplasm. Again the possibility is not excluded that compounds leached out by the rinsing are responsible for a change in the pectins of the cell-wall.

BRIGGS (1958) who also worked with beetroot disks calculated the relative volume of the Donnan phase at 2,1 %. He calculates that if the immobile anions are fixed to surfaces, the cytoplasmic surface is not sufficient to bind all the cations of the Donnan phase. On the other hand the area of the microfibrils of the cell-wall might be sufficient. But because it is known that the cytoplasm contains proteins which may ionise to give immobile anions, Briggs ignores the cell-wall and locates the bulk of the Donnan phase within the cytoplasm. Briggs arguments are not very convincing for it is known that the cell-wall contains pectins which might act as immobile anions. Briggs second argument is based on the osmotic pressure of the Donnan phase. If the concentration of the immobile anions is 600 μeq/l, and the counterions monovalent, the Donnan phase would have an osmotic pressure of about 11 atmospheres, provided that the activity of the counterions was similar to that in aqueous solution. The osmotic pressure of the vacuole is also about 11 atmospheres and because the cytoplasm is in osmotic equilibrium with the vacuole, this would indicate that the Donnan phase is located in the cytoplasm. Though this seems to be a strong argument, we saw that in general the data in favour of a location of the Donnan phase in the cytoplasm are not very convincing.

It has been demonstrated in chapter v that the Rb-ions of the fraction B can be transferred to fraction C and that this process can be inhibited by monoiodoacetamide.

Data about the location of the fraction B will, therefore, give information about the location of the accumulation mechanism by which ions are transferred from B to C. Since in literature opinions differ on the site of the exchangeable cation fraction it seemed worth-while to find out the location of the fraction B in *Vallisneria* leaves. As the establishment of the exchangeable fraction is completely independent of metabolic activity, it could be expected that the Rb fraction B, if situated in the cell-wall, will also be formed when the cell-wall is separated from the cell.

In the following way the exchangeable ions in isolated cell-wall material were determined. In order to separate the cell-wall from the cytoplasm and vacuole, *Vallisneria* leafstrips were homogenised for 3 minutes in a Bühler homogeniser at a speed of 25000 rotations per minute. Next, the cell-wall material was precipitated by centri-

fuging (100 g) and washed thoroughly. It could be shown microscopically that after centrifuging about 90 % of the cells are optically empty. The rest of the cells were not destroyed by the homogeniser. The percentage of organically bound nitrogen in the precipitate as determined by the micro-Kjeldahl method, (LOOMIS and SHULL, 1937) amounted to 10 % of that of the living leaf. This is in good agreement with the percentage of the non emptied intact cells present in the isolated cell-wall material.

The cell-wall material of a number of sets was suspended in a Rb*Cl solution and precipitated after 60 minutes. The cell-wall samples were next rinsed in deionised water or bathed in an unlabelled RbCl solution. From this the fraction (B + C) and C could be determined as described in chapter I, for intact leaves. During the isolation of the cell-wall material, a fraction, equivalent to four leafstrips each measuring 2,5 × 4 mm, was always lost.

For this a correction has been made. Besides a correction for the loss of cell-wall material also a correction was made for the percentage of intact cells in the precipitate.

When W represents the actual % of Rb* in the cell-wall and E is the % experimentally found

$$W = \frac{10}{9} (E - 10).$$

The results are shown in table 7.

TABLE 7

Rb* uptake by the isolated cell-walls from 4 sets Vallisneria leafstrips. Fraction B in percents of the exchangeable fraction of intact leaves.

Data obtained in different experiments	Average	After correction for 0,5 set	After correction for intact cells
36			
47			
50			
40	41	47	41
40			
23			
45			
49			

From these experiments the conclusion can be drawn that only 40 % of the fraction B can be located in the cell-walls. This would mean that the other part has to be located somewhere in the cytoplasm, if at least the method is completely reliable. However, in chapter IV it has already been discussed that it is difficult to determine the maximal size of the exchangeable cation fraction by means of a monovalent cation. If other monovalent cations and especially divalent cations are present they will interfere strongly with the Rb-ions. The degree of competition depends on the concentration of the interfering cations.

For technical reasons it was not possible to keep the concentration of these interfering cations constant neither during the determination of the size of the fraction B in intact cells nor during the determination of the fraction B in isolated cell-wall material. The volume of the salt solution in which the cell-wall fraction was suspended, was much smaller than the volume in which the intact cells were bathed. Thus, an equal quantity of exchanged ions from the material would attain a higher concentration in the former solution.

It is conceivable, therefore, that the measured low exchange capacity of the cell-wall for Rb is due to a higher concentration of interfering ions. Though this has not been experimentally checked, it throws doubt upon the validity of the value arrived at. A trivalent ion would, at least if it could enter the Donnan Space, practically not be influenced by interfering monovalent and divalent cations because of its much higher charge. That is why the trivalent Lanthanum ion has been used to ascertain the exchange capacity of the cell-wall.

In preliminary experiments with unlabelled Lanthanum it was established that Lanthanum can enter the Donnan Space for it was found that it could remove Rb from fraction B by exchange. However, if the Donnan Space had previously been filled with Lanthanum it is very difficult to exchange the Lanthanum ions for Rb ions: it was found that a fraction B did not arise when these Lanthanum pretreated leaves were bathed in a RbCl solution at a high concentration (.01 M). for as long as one hour. Lanthanum is not injurious to *Vallisneria* leaves, provided it is not administered in too high a concentration and for too long a time. It should be noticed that the criterion for this, viz. infiltration of the intercellular spaces, is a very sensitive one. The determination of the percentage of the La fraction B that is present in the isolated cell-wall was carried out in a way similar to that for Rb. First the La fraction B in intact cells was determined.

Then the quantity of Lanthanum in the isolated walls of an equal number of leaf strips was measured. The uptake of labelled Lanthanum in intact leafcells (black dots) and the decrease in the leaves of labelled Lanthanum by exchange against unlabelled Lanthanum are shown in Fig. 28 (circles). After 24 hours the uptake of Lanthanum was still going on.

The small rate indicates that it had nearly come to a stop. The curves represent the sum of the fraction B and C. It must be assumed that fraction C is small. Firstly, the exchange curve (open circles) shows that after 19 hours a small quantity of labelled Lanthanum remained in the leaves. By that time the exchange was still proceeding, but the experiment had to be cut short because infiltration of the intercellular spaces began. Thus, only a part of the small quantity of labelled ions left after 19 hours will have belonged to fraction C. A second reason for assuming that the fraction C is small for Lanthanum is the fact that this fraction is small for the bivalent Ca. It is very likely that with trivalent Lanthanum fraction C is still

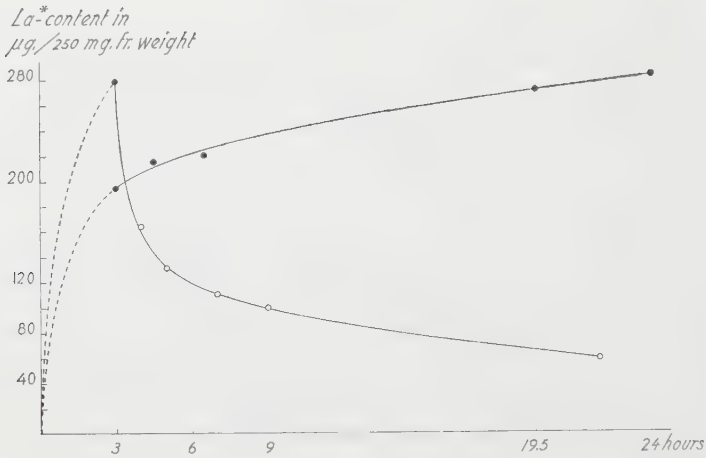

Fig. 28. The course of lanthanum absorption and exchange in intact leaves. ●——● La uptake from a .00186 M La*Cl_3 solution after various periods. ○——○ labelled La content after exchange of the La* absorbed within the first three hours for unlabelled La. Each point represents the average value of two replications.

smaller. Therefore, it can be safely assumed that the whole uptake of La by Vallisneria leaves is adsorption-exchange (Fraction B).

The method of preparing the cell-wall material was the same as described above for Rb except that homogenizing and washing was carried out at a rather low temperature (0° C–10° C) and in the presence of an acetate buffer pH 4,2. (Jansen, *et al.*) in order to prevent a possible deesterification of the pectins in the cell-wall. According to Keller and Deuel (1957) these pectins are responsible for the adsorption of the greater part of the fraction B. In the course of the experiments it was found that neither temperature nor the use of an acetate buffer had any influence on the size of the Lanthanum fraction B, i.e. on the exchange capacity. The washed precipitate

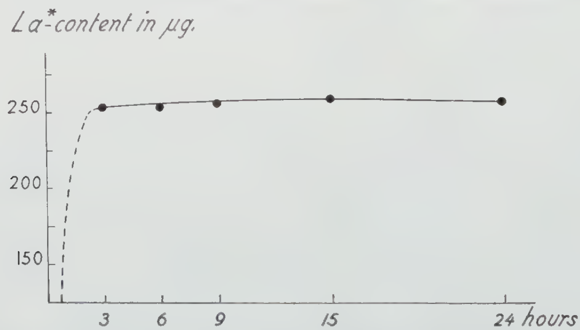

Fig. 29. The course of La* absorption by isolated cell-walls. The isolated cell-wall material is obtained from 1 g fresh weight leaf material. Each point represents the average value of two replications.

was next suspended in a labelled .00186 M $LaCl_3$ solution and the size of the fraction B determined after different incubation periods. The results are shown in Fig. 29. It appears that after an incubation period of 3 hours the size of the La fraction B in the cell-wall homogenate remains constant.

Thus it was sufficient to bathe cell-wall homogenate during three hours in $LaCl_3$, whereas intact cells must be in contact with the La ions during 24 hours. The results are given in table 8.

The percentage of Lanthanum found in the isolated cell-wall material increases with the number of leaves used. In Fig. 30 this percentage has been plotted against the reciprocal of the number of leaves used in each experiment. The points fit a straight line that can be described by the equation. $Y = K - \frac{A}{x}$.

In this equation Y stands for the amount of Lanthanum found in isolated cell-walls as percentage of Lanthanum in intact leaves, x for the number of leaves used; K and A are constants.

Presumably, A represents a definite quantity of cell-wall material

TABLE 8

La* uptake by the isolated cell-walls of Vallisneria leaves in percents of the uptake of La* by intact leaves

Number of sets	La* uptake in percents of the La* uptake by intact leaves
2	76
3	84
4	86
6	89
8	91

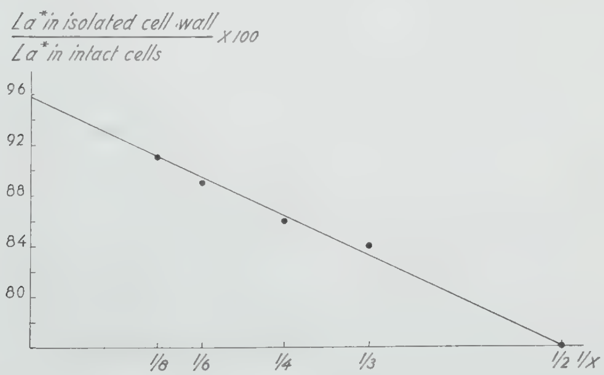

Fig. 30. The percentage of the La* fraction B in intact cells present in isolated cell-walls in dependency of the amount of leaf material taken for the preparation of cell-wall material. × represents the number of sets. Each point represents the average value of two replications.

that is lost during the preparation independent of the number of leaves used. When the number of leaves used is increased the factor $\frac{A}{x}$ decreases and the value for the percentage of Lanthanum in the cell-wall approaches to K. The intercept of the curve and the ordinate gives the value of K when an infinite number of leaves would have been used in the preparation of the cell-wall material. This value amounts to 96 %. This means that practically the whole Lanthanum fraction B is present in the cell-walls. One may wonder whether the same holds for the Rb-ions. We saw that it was only possible to demonstrate that at most 40 % of the Rb fraction B is in the isolated cell-wall. However, it was possible to exchange the whole Rb fraction B for La ions. From the result follows that if the La fraction B is located in the cell-wall, the Rb fraction B too must be located in the cell-wall.

2. The location of the Rb fraction A and its nature

In chapter II it has been shown that a fraction A originates in the cells of *Vallisneria* leaves, when these leaves are bathed in a solution of RbCl. The fraction A is given off again, when the leaves are next bathed in deionised water. The ions of the fraction A are therefore present in a compartment into which they easily enter by diffusion. If this compartment is identical to what usually is called a free space, the concentration of the ions in this compartment is the same as in the surrounding solution, and the size of the fraction A must be linearly proportionally to the concentration of the external solution after establishing of the equilibrium. However, it is not possible to prove this experimentally with Rb-ions. The size of the Rb fraction A was small as compared with the Rb fraction B and as has been discussed in chapter 3, this makes it difficult to determine the Rb fraction A with sufficient accuracy.

A linear relationship of the fraction A to the concentration of the external solution could be shown for the anion SO_4 (Fig. 31). This result and the fact that the ions are lost to deionised water, indicate strongly that the ions of the fraction A are actually present in a free space. Calculation of the size of the free space from the size of the SO_4 fraction A and the volume of the leaves, gives a free space equal to about 6–7 % of the volume of the leaves.

The size of the free space in *Vallisneria* leaves has been also determined by Kylin (1957). From his data a free space of the same magnitude could be calculated. One may wonder whether the fraction A is situated within the leaves, because it is also possible that it must be ascribed to the adhering liquid, which remains on the leaves after blotting.

Though the adhering salt solution surely screens an exact determination of the size of the fraction A, the possibility can be excluded that it constitutes the total fraction A, as the SO_4 fraction A reaches its maximum size only after 5–10 minutes (Kylin, 1957).

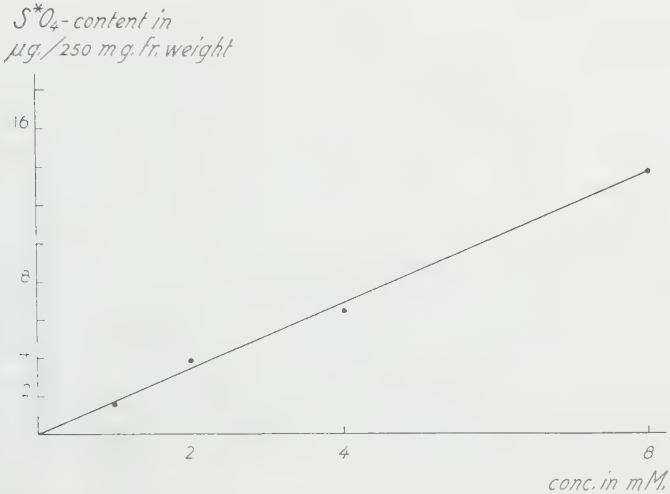

Fig. 31. The size of the S^*O_4 fraction A after an one hour uptake from various concentrations of $K_2S^*O_4$. Each point represents the average value of four replications.

KYLIN (1960) obtained still another argument indicating that the fraction A has not to be considered as an artefact. He actually showed that the moss *Thuidium*, which has a surface area of about 700 cm^2/g fresh weight has a free space which is larger than that of the moss *Plagiothecium*, though this moss has a surface area of about 1000 cm^2/g fresh weight. The free space of roots, called A.F.S. by HOPE and STEVENS (1952) is considerably higher than that of *Vallisneria* leaves and lies between 20 and 33 % of the root volume (BUTLER, 1953; EPSTEIN, 1955). Because the quotient $\frac{\text{surface}}{\text{volume}}$ is higher in roots than in *Vallisneria* leaves, in roots a microscopic thin liquid film will strongly screen the size of the free space (A.F.S.) and the real free space will be much smaller (LEVITT, 1957). On the contrary it is possible that the blotting of leaves affects the size of the fraction A in the reverse direction, by absorption of a part of the ions from the free space.

Opinions on the location of the free space depend on opinions on the place of the membrane, which forms a barrier to the free diffusion of ions into the plant cell. If the tonoplast is considered as the semipermeable membrane, the free space is located in the cell-wall and the cytoplasm, (HOPE, 1953; HYLMÖ, 1953; EPSTEIN, 1956) and BRIGGS *et al.* 1958) but if on the contrary the outer boundary of the cytoplasm is regarded as the semipermeable membrane, the free space is restricted to the cell-wall (PLOWE, 1931; DAVSON and DANIELLI, 1943; WALKER, 1955; LEVITT, 1960).

With the aid of the electron microscope it has been shown by SJÖSTRAND (1954) that in meristematic cells there is a structural difference between the outer boundary of the protoplasm and the

rest of the protoplasm. It gives the picture of a simple unperforated membrane of 50–100 A thick, which is not of the same type as the double membranes of plastids, prctoplastids or chondriosomes. This worker considers the plasmalemma as an artefact. One should keep in mind, however, that a difference in structure does not implicate a difference in physiological behaviour, so that it is not allowed to conclude that such an outer boundary is also a barrier to free diffusion of ions. Buvat and Lance (1957) showed electron-microscopically that the cytoplasm of leaves of various plants is bordered by a double membrane. It is known that this kind of membranes generally forms barriers to the free diffusion of ions. In the previous section on the location of the fraction B in the plant cell, much evidence has been obtained in favour of a location of the total fraction B in the cell-wall. This implicates that the fraction A must also be restricted to the cell-walls, for otherwise a part of the fraction B would be located in the cytoplasm too, owing to the presence of immobile anions.

CHAPTER IX

GENERAL DISCUSSION

The presence of an exchangeable anion fraction

In chapter II it has been shown that an anion fraction B, if present, must be very small. According to Hope (1953) this is intelligible because at normal pH's the acid dissociation of amphoteric groups strongly predominates in plant cells. Also in literature there is much evidence for the existence of an exchangeable cation fraction in plant tissues but little for an exchangeable anion fraction (Kylin and Hylmö, 1957; Leggett and Epstein, 1956). This is in accordance with the present experiments. Yet there are some reports of an exchangeable anion fraction.

Epstein (1955) found in barley roots a small labile bound SO_4 fraction. Selenate ions proved to compete with the SO_4 ions for the binding-sites and for this reason, this SO_4 fraction has been considered to be due to adsorption-exchange (Laties, 1959). Because it could be washed out in deionised water, we must accept that it is exchanged either for the hydroxyl ions, which are present in a very small concentration or for other anions in the medium. These anions may have diffused out of the plant tissue.

Overstreet and Jacobson (1946) found in barley roots an exchangeable phosphate fraction but there was a marked difference in behaviour between the exchangeable Rb fraction and the exchangeable phosphate fraction in regard to their exchange for the unlabelled isotope. Whereas the Rb ions were efficiently removed, the phosphate ions were only slowly removed at a constant rate.

They placed the roots at a temperature of zero degree centigrade in a salt solution of the carrier-free iodine. By this the specific activity was very high and uptake and exchange of very small fractions

could be measured. The concentration of the iodide ions was very low, about 10^{-9} M. Also here a marked difference in the behaviour of the anion and the cation was found. Whereas the uptake of Sr was very little affected by temperature and by killing the roots by ether, the uptake of the I^- was markedly reduced. Moreover the exchange of the absorbed iodide for the unlabelled isotope was very slow and almost linear to time in contrary to the normal exchange curve of an exchangeable cation fraction. Their conclusion was that the exchangeable iodide fraction is due to a metabolic uptake in contrast to the exchangeable Sr fraction, which is non metabolic. Anyhow, the nature of this exchangeable iodide fraction is quite different from the nature of the cation adsorption-exchange fraction and, therefore, cannot be compared with the latter.

LUNDEGÅRDH (1958) assumes that the "Initial Chloride Uptake" found in excised roots is based on adsorption and not on diffusion. This adsorption may be compared with the adsorption-exchange of cations. He claims that the fact that the initial absorption of chloride could be inhibited by KCN points to a subordinate role of pure diffusion in the initial uptake of salts. Lundegårdh, however, only determines differences in the concentration of the external solution before and after the uptake period. In this way adsorption-exchange cannot be demonstrated and experimental evidence for his conception was not obtained.

CARRIERS

As has already been mentioned in chapter I one of the theories about the mechanism of ion absorption by living cells is the carrier theory. This theory starts from the hypothesis that the ions are bound to some movable compound of the cytoplasm, which can pass a membrane impermeable to free ions. It may be asked whether or not the exchange sites to which the cations of fraction B are attached are identical with these carriers. By the experiments of several investigators EPSTEIN and HAGEN (1952), SCOTT and HAYWARD (1954), EPSTEIN and LEGGETT (1954), it has been shown that a very specific competition exists between ions of the same charge and between different groups of ions. Therefore, it is believed that special carriers react with particular ions. However, in *Vallisneria* there proved to be practically no specificity of the exchange sites for monovalent cations (chapter IV). This result is not in favour of the opinion that the exchange sites of fraction B are carriers in the above mentioned sense (LUNDEGÅRDH 1958).

A second objection is that the number of exchange sites is very large. This is in contrast with the generally accepted idea that carriers are present in very small amounts (HAGEN and HOPKINS, 1955; HAGEN, LEGGETT and JACKSON, 1957). The third objection is that the amount of anion carrier would be very small as opposed to the amount of the cation carriers. The most convincing proof against the supposition was obtained in the present experiments in which it

was demonstrated that the adsorption exchange (Fraction B) is not a necessary step in the metabolic uptake of the cations.

We may, therefore, safely assume that the adsorption-exchange in *Vallisneria* has nothing to do with reversible binding to carriers as described by the supporters of the carrier theory.

The location of the exchangeable fraction

It has been concluded in chapter VIII that 96 percent of the La fraction B is located in the cell-wall. However, in calculating the percentage of the La fraction B in the cell-wall, the starting point was that the whole uptake of La-ions in *Vallisneria* leaves is non-metabolic, viz. fraction B. Though it has been discussed in chapter VIII that a metabolic dependent La uptake, if present, must be very small, the possibility that a small metabolic dependent La uptake is present, can not be fully excluded. This possible error would decrease the calculated percentage of the La fraction B in the cell-wall. On the other hand we have seen that the La uptake in the living cell has not completely stopped after 24 hours. This may affect the result in the opposite direction. Both errors are small and they will not influence the result very much. Another objection against the conclusion that the La fraction B is in the cell-wall, may be based on the possibility that by homogenizing the tissue, the cell-wall is not completely separated from the cytoplasm. A measure for cytoplasm in the homogenate is its nitrogen content. The cytoplasm in the cell-wall after preparation is, at least partly, present in intact cells. These cells constitute a certain percentage of the total number originally present in the leaves. This percentage was determined by counting. It was approximately equal to the percentage of the nitrogen of the intact leaves that had remained in the cell-wall. This indicates that apart from the intact cells, little cytoplasm was present.

A second indication that the La fraction B is really located in the cell-wall, follows from the result, that the La fraction B in isolated cell-wall from plasmolysed cells has the same size as the La fraction B in isolated cell-wall from unplasmolysed cells. It is very unlikely that in plasmolysed cells the whole surface layer of the cytoplasm adheres to the cell-wall. Therefore, it is concluded that the exchange sites for the La ions are located in the cell-wall.

The transfer of Ca from fraction B to fraction C

In chapter VII it has been shown that the Ca-ions of the fraction B are hardly transferred to fraction C, whereas a metabolic Ca absorption occurred if the leaves were bathed in a salt solution of $CaCl_2$. This metabolic uptake of Ca into fraction C could be maintained for at least 24 hours. It may be asked why transfer of Ca from B to C does not take place, whereas Rb is readily transferred. As ions can be obtained from fraction B by exchange for other ions only, the transfer from B to C must depend upon exchange. It has been discussed in chapter IV that exchange of divalent cations of the D.F.S. for

monovalent cations is difficult, whereas the monovalent rubidium ions are readily exchanged for other monovalent ions. If this is true we may conclude that the transfer of ions from fraction B to fraction C depends upon exchange of these ions for monovalent cations (probably hydrogen ions) from the cell.

The transfer of Ca from fraction A to fraction C

It was concluded from the experiments in chapter VII on the Ca uptake that the metabolic Ca uptake into the fraction C occurs directly from the external solution (fraction A) and not via the Ca fraction B. This has been considered to be a strong indication that the same holds for the Rb uptake into the fraction C.

One may wonder whether this is justified. In the following respect uptake of rubidium is similar to the uptake of calcium. The uptake into C of both ions is an active process, which may be inhibited by the same metabolic poisons. The fraction B of both cations is present in the cell-walls, since both may be exchanged for lanthanum and since 96 % of the lanthanum fraction B is located in the cell-walls. Neither a calcium fraction B nor a rubidium fraction B has been demonstrated to be present in the cytoplasm. This means that the active uptake into C takes place at the outer boundary of the cytoplasm. The only difference is that rubidium may be transferred from A to C and from B to C, whereas calcium is only transferred from A to C. There is no reason whatsoever to assume that a calcium fraction A may penetrate beyond the outer layer into the cytoplasm whereas a rubidium fraction A would stop at that layer. The fate of the two cations in the cell may be different, but it is not relevant to the question considered here. Whereas the Rb-ions are probably mainly accumulated into the vacuole (A. VAN SCHREVEN and A. VAN DER MOLEN, ARISZ in 1943, 1956) the Ca presumably remains in the cytoplasm (MAZIA, 1938), though it is also known of Ca ions that they may accumulated in the vacuole (CHASSON and LEVITT, 1957). We can therefore safely assume that the conclusion drawn from the experiments with $CaCl_2$, that the exchangeable fraction is not necessary for irreversible uptake, also holds for the Rb uptake into the fraction C.

The location of the metabolic uptake process in the cell

From previous researches it has appeared that the uptake of chloride into the cytoplasm of *Vallisneria* leaves is an active process, using energy available in the cell or supplied when exposed to light (ARISZ, 1947, 1952, 1956). In chapter III it has been shown that the rate of the Rb uptake into the fraction C can strongly be inhibited by inhibitors of metabolic processes.

Moreover it was shown that the rate of uptake was dependent on temperature. From these results it may be concluded that the cation uptake into the fraction C is also only possible by an "active" accumulation mechanism. However, according to LUNDEGÅRDH (1945) the possibility remains that the rate of the cation accumulation in

cells is only inhibited by such factors as poisons, temperature etc., because the uptake of the accompanying anion is inhibited by these factors. The cation accumulation in it self may be a non-metabolic process, so that the cations are dragged passively with the anions, in order to maintain electrical neutrality within the cell. Whether the Rb-ions are passively dragged into the cells by anions which are taken up actively, or taken up actively is irrelevant to the problem discussed here.

In chapter v it was shown that not only the uptake of Rb into C is a metabolic process but also the transfer from B to C. It could be inhibited by monoiodoacetamide and it had a high temperature quotient. During the transfer of the Rb from fraction B to fraction C no added anions are available to join the Rb-ions. A fraction A has been washed out in the deionised water (chapter v) and for the anion a fraction B does not exist in measurable amounts (chapter II). Because in several experiments the pH of the deionised water was held at a pH 5, it is unlikely that measurable amounts of bicarbonate ions, that might join the Rb ions during the transfer to the fraction C, were present in the deionised water. These findings are strong indications that an uptake of cations exists which is independent of the uptake of anions. It follows that the cation uptake can directly be dependent on metabolism and that the outer boundary of the cytoplasm forms the barrier for a free diffusion of ions, which can only be passed at the expense of energy.

Arisz demonstrated (1956) that cyanide has not a direct inhibiting influence on the secretion into the vacuole, but that it inhibits a process by which ions are actively absorbed into the cytoplasm. This shows that two different mechanism for the metabolic uptake of chloride are present in *Vallisneria* leaves. Arisz located one mechanism in the tonoplast and the other one somewhere in the cytoplasm. The present experiments do not permit any conclusion with regard to the accumulation mechanisms located at the tonoplast, but they furnish data on the existence of a mechanism regulating the absorption into the cytoplasm and moreover they allow conclusion about the place where this mechanism is located.

Robertson *c.s.* (1955) assume that the accumulation mechanism is not located in the plasmalemma but in the outer boundary of mitochondria. According to Robertson (1957) it is possible that these mitochondria move around by the protoplasmic streaming in the cell and that many of them frequently come into contact with the surface of the vacuole. It would be equally possible that they come into contact with the cell-wall, pick up ions here and lose them to the vacuole. However, it is known that in *Vallisneria* leaves the protoplasmic streaming comes to a standstill (Jager, 1958) under conditions of high metabolic uptake and is not restored for the first few hours after bringing the leaves to deionised water. Thus a transfer of ions from wall to cytoplasm by means of mitochondria is not very likely. But even when it should take place, the first accumulation mechanism must be located at the boundary of cytoplasm and cell-wall.

SUMMARY

1. Cations absorbed by *Vallisneria* leaves could be separated into three fractions, viz:
1. a fraction which was washed out in deionised water (fraction A)
2. a fraction which can be removed by exchange (fraction B)
3. a fraction which can be neither washed out nor exchanged.

2. Absorbed anions could be separated into two fractions only, viz: a fraction A and a fraction C; an exchangeable fraction being absent (chapter II).

3. The formation of the exchangeable fraction B depends on the physico-chemical and that of the fraction C on the biochemical properties of the plant (chapter III).

4. It is concluded that a Donnan equilibrium determines the exchangeable fraction B (chapter IV).

5. The transfer of rubidium ions from the exchangeable fraction B to fraction C is strongly affected by temperature and is inhibited by monoiodoacetamide (chapter V).

6. Of each number of rubidium ions accumulated as fraction B (exchangeable fraction) one part is more readily transferred to C than the rest. The rate of transfer of the first part is approximately linear to the number of ions present in B. The second portion is transferred at a much lower rate (chapter VI).

7. In contrast to exchangeable rubidium-ions, exchangeable calcium-ions of the fraction B are practically not transferred to fraction C, though Ca-ions are taken up in C from the external solution. This uptake depends on metabolism. It is concluded that adsorption-exchange in fraction B does not constitute a necessary link in the uptake of cations from the external solution into fraction C (chapter VII).

8. It is made probable that the exchangeable fraction (fraction B) is located in the cell-wall, and that the metabolic process which accumulates cations into the fraction C is located at the outer boundary of the cytoplasm. (Chapter VIII and General Discussion).

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REVISION DER NIEDERLÄNDISCHEN ARTEN DER GATTUNG AGROPYRON

(VORLAUFIGER BERICHT)

ALFRED HANSEN

(*Botan. Museum, Kopenhagen*)

(*eingegangen am 31. März 1961*)

Die bisher in die Gattung *Agropyron* s.l. eingegliederten Arten Nordeuropas (spontane sowie adventive) sind den neuesten Auffassungen nach (u.a. HYLANDER, 1953) auf folgende Gattungen zu verteilen:

Gattung ROEGNERIA:
(*R. canina* (L.) Nevski; in Nord-Skandinavien und Schottland noch weitere Arten).

Gattung ELYTRIGIA:
(*E. juncea* (L.) Nevski in der ssp. *boreoatlantica* (Sim. & Guin.) Hyl. = *Agropyron junceum* (L.) PB.
E. pungens (Pers.) Tutin = *Agropyron litorale* (Host) Dum.
E. repens (L.) Nevski = *Agropyron repens* (L.) PB.
Dazu noch 3 Hybriden: *E. juncea* × *repens* = "*Agropyron acutum* (DC.) Buch.", *E. juncea* × *pungens* = "*Agropyron obtusiusculum* Lange" und *E. pungens* × *repens*).

Gattung AGROPYRON:
(nur adventive Arten, u.a. *A. cristatum* (L.) Gaertn.).

Die Gattung *Agropyron* s.l. in den Niederlanden ist u.a. in folgenden neueren Florawerken und Abhandlungen ausführlich behandelt worden: JANSEN & WACHTER 1933, JANSEN 1951 und HEUKELS/VAN OOSTSTROOM 1956. Diese Autoren zählen folgende Arten und Hybriden auf (HEUKELS/VAN OOSTSTROOM 1956 hat jedoch schon die Spaltung in die Gattungen *Roegneria* und *Agropyron* vorgenommen):
A. caninum, *A. repens*, *A. junceum*, *A. littorale*, *A. maritimum*, *A. cristatum*. *A. junceum* × *repens*, *A. junceum* × *maritimum*, *A. junceum* × *littorale*, *A. littorale* × *maritimum*, *A. littorale* × *repens*, *A. maritimum* × *repens*.

Dank der sehr grossen Freundlichkeit der Herren Th. J. Reichgelt und Dr. S. J. van Ooststroom, Rijksherbarium, Leiden, habe ich 1956 die Gelegenheit gehabt, rund 550 Herbarbogen *Elytrigia*-Materials aus dem Leidener Herbar (Material ausschliesslich aus den Niederlanden stammend) durchzusehen und zu überprüfen. Das Material stellt ohne Zweifel eine ausreichende und repräsentative Sammlung der in den Niederlanden vorkommenden Arten und Hybriden dieser

Gattung vor. Ich habe selbst das Material aus den Sammlungen in Leiden herausgesucht, und alle mir beim ersten Blick fraglich erscheinenden Bestimmungen wurden danach für nähere Prüfung mitgenommen.

Es ergaben sich folgende Resultate:

1) Die Hybride *Elytrigia juncea* × *pungens* hat in dem "Waddendistrict" und dem "Duindistrict" der Niederlande — wie es nach den Verhältnissen in den Nachbarländern Nordwest-Deutschland und Südwest-Dänemark zu erwarten war (Hansen 1955, 1960) — eine weit grössere Verbreitung als früher angenommen. 209 Stück der Herbarbogen enthalten diese Hybride. Sie stellt wahrscheinlich die häufigste *Elytrigia*-Hybride der Niederlande vor.

2) Auch die Art *Elytrigia pungens* hat in denselben Distrikten in den Niederlanden eine weit grössere Verbreitung, als man sich früher klar war. Insgesamt 202 Stück der Herbarbogen zeigten diese Art.

3) Die Varietät "*lolioides*" von *E. pungens* (*A. littorale*) — von Jansen 1933 aufgestellt — hat kaum systematischen Wert. Sämtliche von mir eingesehenen Herbarbogen dieser "Var." beziehen sich auf die Hybride *E. pungens* × *juncea*.

4) Die von Jansen 1933 aufgestellte Art *Agropyron maritimum* hat gleichfalls kaum systematischen Wert — sie bezieht sich auf entweder *E. pungens*, *E. pungens* × *juncea* oder *E. repens*.

5) Als Konsequenz des Wegfalls von *A. maritimum* müssen auch die von den niederländischen Autoren aufgestellten Hybriden *A. junceum* × *maritimum*, *A. littorale* × *maritimum* und *A. maritimum* × *repens* ausscheiden. Die Überprüfung der mit diesen Namen versehenen Herbarbogen hat auch diese Vermutung bestätigt. Die Hybride *A. junceum* × *maritimum* bezieht sich auf die Hybride *E. juncea* × *pungens*, die Hybride *A. littorale* × *maritimum* auf entweder *E. pungens*, *E. juncea* × *pungens* oder *E. pungens* × *repens*, und endlich bezieht sich die Hybride *A. maritimum* × *repens* auf entweder *E. repens* oder *E. juncea* × *pungens*.

6) Die Hybriden *E. juncea* × *repens* und *E. pungens* × *repens* (11, bezw. 10 Herbarbogen gesehen) sind in den Niederlanden als recht seltene Pflanzen zu betrachten.

7) Die Art *E. repens* stellt in den Niederlanden wie überall eine sehr vielgestaltige Art vor, u.a. mit einer oder mehreren Strandformen oder -Varietäten. Wie diese zu bezeichnen sind, ist vorläufig unklar; die Bezeichnung var. *maritimum* Koch et Ziz ist gar nicht zuverlässig, da die von Koch & Ziz im Jahre 1814 aus dem Binnenland beim Flusshafen Mainz in West-Deutschland beschriebene Pflanze ("in sabulosis prope Moguntiam cum aliis plantis salinis copiose occurrit") sich höchster Wahrscheinlichkeit nach auf eine vom Nordseeküstengebiet her eingeschleppte *Elytrigia pungens* bezieht. Eher sollte der Name var. *littorale* Bab. benutzt werden.

Kurz gefasst enthält die Gattung *Elytrigia* (früher Gattung *Agropyron* Sektion *Elytrigia*) in den Niederlanden folgende Arten und Hybriden: *E. juncea* (ssp. *boreoatlantica*), *E. pungens* und *E. repens*. *E. juncea* × *pungens*, *E. juncea* × *repens* und *E. pungens* × *repens*.

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THE INFLUENCE OF BLUE, RED AND FAR RED LIGHT ON GEOTROPISM AND GROWTH OF THE AVENA COLEOPTILE

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CONTENTS

ABSTRACT

The influence of short lasting pre-irradiations from above with monochromatic light on the geotropic reaction of the *Avena* seedling is dependent on wave length and quantity of the radiation used, on the interval of time inserted between pre-irradiation and unilateral exposure to gravity (termed "waiting time"), and on the length of the period during which the plants are kept in the horizontal position (termed "exposure time").

With red or far red pre-irradiation merely an increase of the geotropic curvature is observed, but only when a "waiting time" of 30 minutes is inserted and the "exposure time" is about 60 minutes.

With blue (or violet) light is observed a decrease in curvature with 1000-10,000 erg cm^{-2}, a "waiting time" of 30 minutes and an "exposure time" of c. 60 minutes, an increase with 100,000 or more ergs cm^{-2}, a "waiting time" of 0 minutes or of 75 minutes and an "exposure time" of c. 60 minutes.

Any preirradiation influences the growth rate of the coleoptile in some way; this applies to plants in the horizontal as well as in the vertical position.

Correlations between growth and geotropic reaction are different in different cases. Correlation between phototropic and geotropic behaviour could not be demonstrated.

No red - far red antagonism could be detected.

CHAPTER I

INTRODUCTION

About fifty years ago A. H. Blaauw (1914, 1915) reduced the problem of the phototropic reaction to the problem of the light-growth response after illumination from all sides. The phototropism occurring after one-sided illumination would be caused by the difference between the light-growth responses of the lighted and the shadow side of the coleoptile. In spite of this early lead the connection between the amount of light energy applied, and the resulting light-growth reaction and phototropic curvature still is not clear. Elucidation of the problem was hampered by the following complications:

a) Different light qualities influence growth and phototropic reaction in different ways.

b) The growth of the mesocotyl is influenced by irradiation with light of any wave length but not in the same way as that of the coleoptile. Therefore it seems opportune to look for connections between phototropic reaction and light-growth reaction of the coleoptile rather than of the whole seedling.

c) Presumably one-sided irradiation has two different effects on *Avena* coleoptiles: the phototropic effect proper and an effect on the phototonus.[1]) These two effects cannot clearly be distinguished when studying phototropism.

Several workers took into account the above mentioned complications. Koningsberger (1922) investigated the influence of a number of wave lengths on the growth of *Avena* seedlings. He measured, however, the growth of the whole plant and therefore supplied no data that could help to solve the problem of the phototropic reaction. Du Buy and Nuernbergk (1929, 1930) measured the growth of the convex and the concave side of the coleoptile, but used white light.

The complication mentioned in c) is distinguished clearly by Arisz (1915). However, he studied this problem using white light. The same

[1]) The term "tonus" is used in this paper as an equivalent of the german "Stimmung". When after a pre-treatment, e. g. by an all-sided preillumination which does not or even cannot (as is the case with red light) cause a phototropic reaction, the phototropic effect of a subsequent unilateral irradiation is altered, this is due to a change of the phototonus. *Mutatis mutandis* the term geotonus is used in the same sense for the geotropic reaction.

was done by BEYER (1927, 1928), PISEK (1928), FILZER (1928) and BREMEKAMP (1915). FRANCK (1951) tried to separate the tropistic from the phototonic effect by allowing coleoptiles irradiated from all sides to curve geotropically instead of phototropically. He also, however, used white light. BLAAUW-JANSEN (1959) investigated the effect of a number of wave lengths on growth and curvation of the coleoptile only, but she irradiated only one side. For that reason, the two effects mentioned in c) always became intermingled.

The present author intends:

1) to work with light "as monochromatic as possible",
2) to irradiate from directly above,
3) to allow the irradiated coleoptiles to curve geotropically,
4) to measure the growth of the curving zone of the coleoptile,
5) to measure the growth of the part of the seedling underneath the curving zone.

The geotropic reaction and the growth thus are used as a measure of the tonic effect of the all-sided illumination.

Experiments, performed according to these principles, may deepen the insight in the mechanism of the phototropic as well as of the geotropic reaction.

CHAPTER II

MATERIAL AND GENERAL METHODS

PLANTS

Husked seeds of *Avena sativa* (cultivar "Siegeshafer") were wetted by shaking them in tap water and put to germinate on moistened filter paper in a closed petri dish in a dark room which was kept at a temperature of 22° C and a relative humidity of about 80 %. In order to suppress mesocotyl growth, the germinating seeds were exposed to orange light for 19 to 20 hours. After this period the seeds were planted in zinc trays filled with moist vermiculite with the embryo pointing vertically downwards and kept in absolute darkness in the dark room mentioned before. At the age of 84 to 85 hours the plants were used for the experiments. In each tray a row of 18 seeds was planted. A short time before the irradiation, all plants that were not straight, or that seemed too tall or too small were discarded. This operation was feasible when working under green light of an intensity of less than 40 erg cm^{-2} sec^{-1}.

LIGHT

The sources of the orange light were incandescent lamps filtered through *Schott OG2* filters.

Monochromatic light was obtained by focussing the light of an incandescent lamp (60 Volt, 3000 Watt) using a condensor lens and filtering the beam through an interference filter made by Balzer

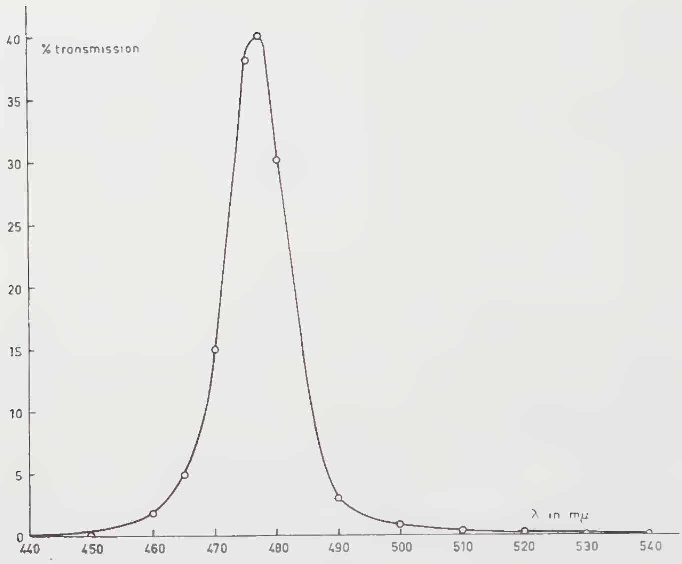


Fig. 1.

(Liechtenstein) of which the tolerance was 0.5 %. A transmission spectrum of one of the interference filters used is shown in Fig. 1.

A mirror reflected the monochromatic light from the filter vertically on top of the plants to be irradiated. As no lens was used to project an image of the condensor lens aperture in the plane of the object, the material was irradiated with a diffused, out of focus image of the light source.

Some of the irregular brightness details of the filament structure of the source were reproduced and the intensity distribution in the irradiated plane was not quite uniform. However, variations never exceeded 15 % and a dependence of the reactions of the irradiated plants on their position in the irradiated plane was never observed. In the light beam a big "*Compur*" shutter was placed.

Whenever the plants were shadowgraphed during the experiments, this was performed with the aid of green light (130 erg cm^{-2} sec^{-1}), produced by filtering a beam of parallel light through an interference filter ($\lambda = 560$ mμ).

The light intensity was measured by means of a thermopile after Moll (Kipp, Delft) and a calibrated Zernike galvanometer (Kipp, Delft).

A summary of the wave lengths and light intensities that were used is given in table 1.

The development and the measuring of curvatures

After irradiation of the coleoptiles with blue, red or far red light the plants were put from the vertical into the horizontal position in such a way that geotropic curvatures would develop in the plane of the

TABLE 1

LIGHT		LIGHT SOURCE		Filter	Light intensity in erg $cm^{-2}sec^{-1}$
Colour	Wavelength in $m\mu$	Watt	Volt		
orange . . .	from 500 upwards	40	220	abs. f.	800
violet . . .	415	3000	60	int. f.	300
blue	479	3000	60	int. f.	900
green . . .	560	100	12	int. f.	40 or 130
red	660	3000	60	int. f.	1400
far red . . .	735	3000	60	int. f.	1850

two vascular bundles. Shadowgraphs were made immediately after the coleoptiles had been placed horizontal and after they had been in this position for 60 minutes. The shadowgraphs were made on Gevaert Document Rapid photographic paper by means of green monochromatic light of an intensity of 130 erg cm^{-2} sec^{-1}. On the shadowgraphs made before any geotropical curvature had developed, the straight and horizontal coleoptiles were selected. Only the curvatures of these selected plants were considered. The curvatures were measured by means of a protractor. Standard errors of the mean, for some experiments, chosen at random calculated by means of the formula

$$SE_m = \pm \sqrt{\frac{\sum (x-\bar{x})^2}{n \cdot (n-1)}}$$

where n = the number of coleoptiles,
$\sum (x - \bar{x})^2$ = the sum of the squared deviations from the mean, seldom amounted to values higher than $\pm$ 2° as shown in table 2.

TABLE 2

Number of coleoptiles	Pre-irradiation	"Waiting time"	Curvature in degrees ("Exposure time" -- 60 min)	Standard error of the mean in degrees
12	9 min 479 $m\mu$	10 min	28	1.3
9	id.	id.	26	3.7
11	id.	id.	28.5	2.1
11	1 min 479 $m\mu$	30 min	12.5	1.8
12	id.	id.	11.5	1.7
13	id.	id.	14	1.1
13	none	—	18.5	1.1
13	id.	—	16	1.6
11	id.	—	16	1.5
9	id.	—	17	1.9
9	id.	—	17	2.6
7	id.	—	20	1.8
11	id.	—	18	1.6
13	4 sec 660 $m\mu$	0 min	26	1.9
12	30 sec 660 $m\mu$	0 min	32	1.6

*) E.g.: "Exposure period" 30–90 min indicates that the plants have been in the horizontal position from the 30th to the 90th minute after the beginning of the irradiation.

Measuring of straight growth

In the zinc trays with vermiculite, in which the seedlings had been cultivated, a hair was stretched along the row of plants approximately ½ cm over the surface of the vermiculite. In addition, as a mark, a particle of vermiculite was fixed on the coleoptile at a distance of about 1 cm from the top by means of a trace of lanoline.

After the coleoptiles had been irradiated with blue, red or far red light, shadowgraphs were made every 15 minutes.

On the shadowgraphs the increase in length of the seedlings was measured from hair to mark and from hair to top, to the nearest 0.1 mm. The increase from hair to mark is the increase in the lower part of the seedling, mainly in the mesocotyl. This increase is referred to as "growth of mesocotyl". The growth of the uppermost 1 cm of the coleoptile, in which region the geotropic curvature develops within 60 minutes, is obtained by subtracting the two values measured. This growth is referred to as "growth of coleoptile". Table 3 gives an impression of the standard-errors of the mean in this type of experiment.

Table 3

Number of coleoptiles	Pre-irradiation	Growth in mm after three hours	
		of mesocotyl	of coleoptile
12	6 sec 660 mμ	1.4 ± 0.11	2.8 ± 0.19
10	6 sec 660 mμ	1.6 ± 0.09	2.3 ± 0.14
10	4 sec 479 mμ	2.4 ± 0.17	1.2 ± 0.09
12	4 sec 479 mμ	2.0 ± 0.13	1.2 ± 0.15
11	dark controls	2.5 ± 0.22	1.3 ± 0.21
10	dark controls	2.6 ± 0.25	1.5 ± 0.17

Measuring of the absolute growth of concave and convex sides of curved coleoptiles

Coleoptiles were marked by a grain of vermiculite approximately 1 cm from the top. These were shadowgraphed before and after the development of a geotropic curvature. On the shadowgraphs α, d, CD and AB were measured as is indicated in Fig. 2.

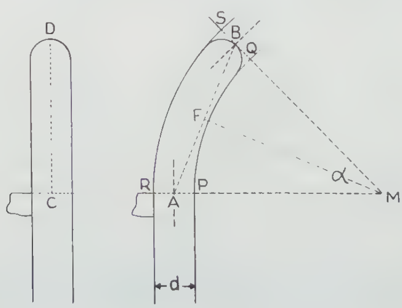


Fig. 2.

Now arc PQ and arc RS can be calculated:

$$\sin \tfrac{1}{2}\alpha = \frac{AE}{AM} \rightarrow AM = \frac{AF}{\sin \frac{1}{2}\alpha} = \frac{AB}{2 \sin \frac{1}{2}\alpha}$$

$$PM = AM - AP = AM - \tfrac{1}{2}d$$

with $d = 1.6$ mm:

$$PM = AM - 0.8$$

$$PQ = \frac{\alpha}{360}\, 2\pi PM = \frac{\alpha}{360}\, 2 \left(\frac{AB}{2 \sin \frac{1}{2}\alpha} - 0.8\right) = \frac{\alpha\pi\, AB}{360 \sin \frac{1}{2}\alpha} - \frac{1.6\,\pi\alpha}{360}.$$

In analogy:

$$RS = \frac{\alpha\pi\, AB}{360 \sin \frac{1}{2}\alpha} + \frac{1.6\,\pi\alpha}{360}.$$

Growth of the convex side = RS — DC.
Growth of the concave side = PQ — DC.

In table 4 the increases in length of convex and concave sides of some sets of coleoptiles, with the standard errors of the mean are shown.

TABLE 4

Number of coleoptiles	Pre-irradiation	Growth in mm after one hour of		Curvature in degrees ("Exposure time" = 60 min)
		concave side	convex side	
12	7000 erg cm^{-2} 660 mμ	0.04 ± 0.03	0.73 ± 0.05	24
9	2700 erg cm^{-2} 479 mμ	0.51 ± 0.05	0.68 ± 0.06	6
10	none	0.10 ± 0.02	0.37 ± 0.02	10

CHAPTER III

INITIAL EXPERIMENTS

a) The necessity of illuminating the seedlings with orange light during the germination period in order to suppress the growth of the mesocotyl and to obtain straight plants is a confusing factor when studying the influence of red and far red light on the geotropic reactions of the coleoptile.

Therefore, the influence of the orange irradiation during the germination on the sensitivity of the coleoptile for red light was examined in experiments 1 and 2.

EXP. 1

The seeds germinated under an orange lamp. They were planted in vermiculite 17, 18, 19, 20, 22 and $23\frac{1}{2}$ hours resp. after having

been put on the filter paper. Consequently, the irradiation period also varied from 17 to 23½ hours.

When the seedlings were 84 hours old part of them was irradiated with red light (2800 erg cm^{-2}), the rest was kept in darkness. Thereupon they were placed horizontal and after 75 minutes the curvatures were compared.

At a rough estimate red light enhanced the geotropic curvature of those seedlings that had been pre-illuminated for 19, 20 and 22 hours.

Exp. 2

The seeds were divided into two groups that were pre-illuminated for 16¼ and 19 hours. Of each group two batches of plants were irradiated with red light, two with far red light and four were kept in darkness.

Subsequently all plants were put horizontal and after 75 minutes their curvatures were measured.

The following curvatures had developed (table 5).

Table 5
Curvatures developed in 75 minutes

Irridiated with	Pre-illuminated for	
	16¼ hours	19 hours
2700 erg cm^{-2} 660 mμ .	24°	23°
12000 erg cm^{-2} 735 mμ .	25°	28°
dark control	20°.5	20°.5
dark control	20°.5	20°

On account of these experiments it was decided to pre-illuminate the seeds with orange light during a 19 hours germination period.

b) It was thought necessary to find the optimal time interval between the illumination and the beginning of the "exposure time" [1]) to unilateral gravity. Therefore batches of approximately 10 plants

Table 6

"Waiting time" in min	Curvature in degrees after irradiation with		Curvature of dark controls in degrees ("Exposure time" = 75 min)
	2800 erg cm^{-2}, 660 mμ	12000 erg cm^{-2}, 735 mμ	
0	31	29	29
30	38	34	26
60	26	26	27
120	29	28	28

[1]) In this paper with "exposure time" is meant: the period during which the plants were kept in the horizontal position.

were irradiated either with 2800 erg cm^{-2} of red light or with 12000 erg cm^{-2} of far red light. Controls were kept in darkness.

The plants were placed horizontal 0, $\frac{1}{2}$, 1 or 2 hours after the irradiation. 75 minutes after that, shadowgraphs were made. Results are shown in table 6.

Consequently for the experiments to come a "waiting time" [1]) of 30 minutes was chosen to start with.

c) An experiment was performed to determine the optimal "exposure time". Red light enhances the geotropic reaction. During the first half hour in which the plants are in the horizontal position no curvature can be observed. After a few hours illuminated as well as non-illuminated plants show curvatures of more than 60°.

The greatest percentual difference in rate of curvation between illuminated and non-illuminated plants can be observed after 60 to 70 minutes.

TABLE 7

A			B		
Quantity of light of 660 mμ in erg cm^{-2}	Curvature in degrees ("Exposure time" = 60 min)		Quantity of light of 735 mμ in erg cm^{-2}	Curvature in degrees ("Exposure time" = 60 min)	
	exp. 1	exp. 2		exp. 1	exp. 2
4	18	24	12	21	18.5
8	21	22	30	20	22.5
16	22.5	23	60	23	25
dark	21	17	dark	16	21.5
43	26	24	150	22	24
86	23.5	22	300	22	23
200	24	22	600	18	23
dark	18.5	17	dark	16	22
400	25	28	1,200	24	26
850	22.5	26	2,500	27	24
1,700	23	26	5,000	25	24.5
dark	17		dark		21
3,500	28	32	10,000	24	26
7,000	30	30	20,000	27	29
13,000	25.5	30	40,000	25	32.5
dark	21.5	20	dark	18	20
26,000	26	32	80,000	31	31
50,000	27	31	160,000	26	
100,000	29	30			
dark	18	18	dark	20	19.5
200,000		31	600,000	29	
400,000		28	600,000	29	
800,000		33	600,000	29	
dark		19	dark	21	

[1]) In this paper with "waiting time" is meant: the interval of time between the beginning of the illumination and the moment at which the plants are placed into the horizontal position.

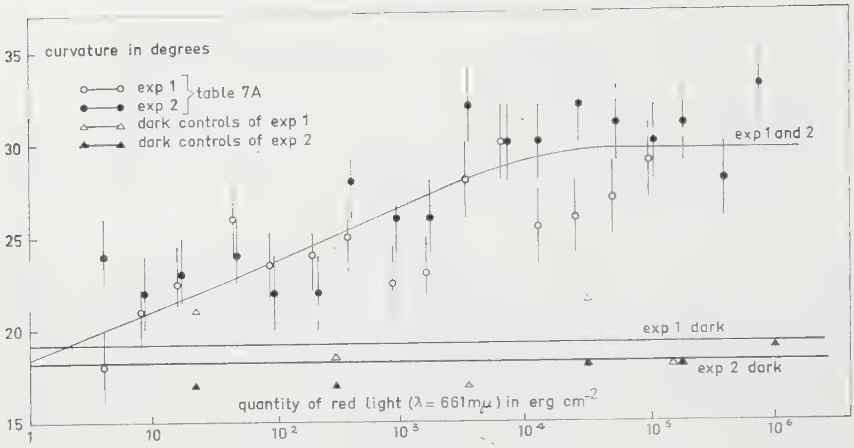


Fig. 3.

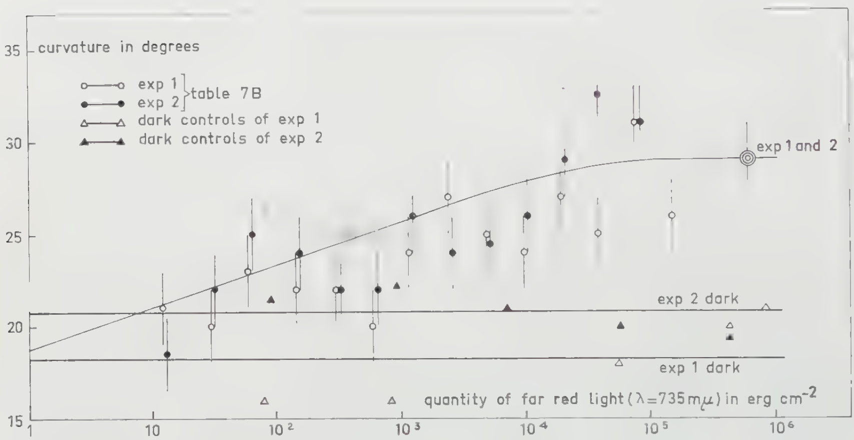


Fig. 4.

This, combined with reasons of convenience, led to the decision to fix the "exposure time" on 60 minutes.

d) In experiments on the influence of red and far red light on the phototropic curvature of the coleoptile (BLAAUW-JANSEN 1959) quantities of 700 erg cm^{-2} of red light and of 6000 erg cm^{-2} of far red light were sufficient to obtain a maximal effect. As it is not beforehand established that the same quantities are required to exert a maximal influence on the geotropic reaction of the coleoptile, a series of trays was irradiated with doses of red and far red light in the range of

4 erg cm^{-2} to 800,000 erg cm^{-2}. This means that the time of irradiation varied from 0.005 sec to 9 min for red, and from 0.005 sec to 5.5 min for far red light. After a "waiting time" of 30 minutes the geotropic curvatures which developed in the next hour were measured (table 7, Fig. 3 and 4). Since the working schemes of these extensive experiments did not allow to administer the irradiations mentioned within a short period of time, it had to be tested whether the quality of the plant material changed during the experiment. For that reason, during the course of an experiment, dark controls which had been horizontal during the same period as the irradiated trays, were measured as a check upon possible aging effects. The values of the dark controls did never indicate any aging effect; it always seemed allowed to represent these values by a straight horizontal line.

Table 7 and Fig. 3 and 4 show that a quantity of about 7000 erg cm^{-2} of red light and about 20,000 erg cm^{-2} of far red light are sufficient to obtain a maximal effect.

Summary of Chapter III

Preliminary experiments are described in which were determined:

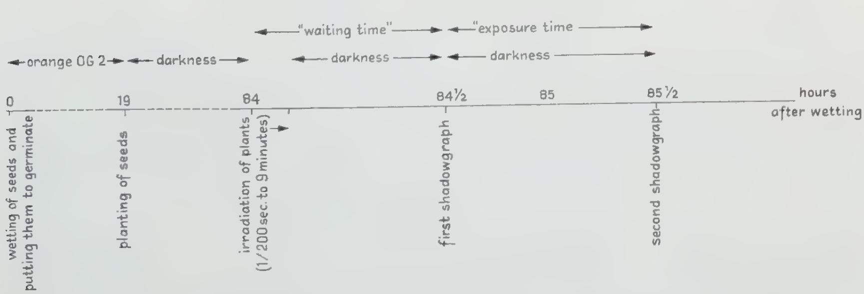
a) The length of the period during which the germinating seeds have to be illuminated with orange light to get a maximal effect on the geotropic reaction (19 hours).

b) The optimal "waiting time" i.e. the interval of time that has to be inserted between the beginning of the pre-irradiation and the beginning of the unilateral exposition to gravity in order to obtain maximal enhancement of the geotropic reaction (30 minutes for red and far red light).

c) The optimal "exposure time", i.e. the period during which the plants should be kept horizontal in order to obtain the greatest difference in the geotropic curvatures between the various treatments (60 minutes).

d) The quantities of red and far red light, sufficient to obtain a maximal effect on the geotropic reaction (7000 and 20,000 erg cm^{-2} resp.).

Working scheme of the experiments from table 7, fig 3 and 4



CHAPTER IV

THE INFLUENCE OF BLUE LIGHT ON THE GEOTROPIC CURVATURE OF THE COLEOPTILE

As a preliminary working hypothesis in this laboratory the phototropic effect of one-sided illumination with blue light is supposed to consist of:

α) a phototonic effect (possibly induced by an all-sided lowering of the indole—3—acetic acid (IAA) level in the coleoptile),

β) the phototropic effect proper (possibly brought about by a destruction of IAA at the irradiated side of the coleoptile or by a shift of the IAA from the irradiated to the shadow side, or by a combination of the two).

The aim of the present author was to separate these two effects (α and β), replacing β by the effect of a geotropic induction and α by irradiation of the coleoptile from right above.

Therefore, coleoptiles were irradiated from above with 20; 10,000 or 50,000 erg cm^{-2} of blue light ($\lambda = 479$ mμ). The experimental working scheme was the same as the one given in the last paragraph of chapter III.

The following geotropic curvatures were observed (table 8):

TABLE 8

Quantity of blue light in erg cm^{-2} ($\lambda = 479$ mμ)	Curvature in degrees ("Exposure time" = 60 min)	
	exp. 1	exp. 2
0 (dark)	17.5	19.0
20	15.7	13.0
10,000	12.0	11.0
50,000	14.5	16.5

A decrease of the geotropic curvature followed by a recovery after irradiation with 10^4 and 5×10^4 erg cm^{-2} resp. is suggested by this experiment, the decrease being perhaps present already with 20 erg cm^{-2}. This effect was proved in experiments of which the results are shown in table 9 and Fig. 5. The same experimental procedure was pursued as in the experiments of table 8,[1]) but a wider and more differentiated range of light quantities was administered. Each figure gives the mean of 12 plants approximately.

Indeed, after a quantity of 1800–3600 erg cm^{-2} of blue light a minimum in the geotropic reaction of the coleoptile occurs.

In table 10 the results of another experiment of the same type are given. This experiment, in which each of the mentioned curvatures

[1]) Working scheme: see p. 407.

TABLE 9

Quantity of blue light in erg cm^{-2} (λ = 479 $m\mu$)	Curvature in degrees ("Exposure time" = 60 min) exp. 1		exp. 2	
$4\frac{1}{2}$	25		16	
9	16		19.5	
22	16		11	
dark		25		16
45	20.5		14	
110	11		10	
225	22		11	
dark		27		18
450	10		9	
900	14		8.5	
1,800	5		5.5	
dark		22.5		20
3,600	13		5.5	
7,000	16		13	
14,000	23		17	
dark		22.5		20
27,000	23		20.5	
55,000	21		21.5	
110,000	29		26.5	
dark		25		
220,000			22	
440,000			22	
440,000			24	
dark				19

TABLE 10
"waiting time" = 30 min
"exposure time" = 60 min

Quantity of blue light in erg cm^{-2} (λ = 479 $m\mu$)	Geotropic curvature in degrees
1800	6
2700	2
3600	4
none	22

is the mean of that of 50 plants approximately, was made to locate the exact position of the minimum of the graph of Fig. 5.

The experiment of table 10 was repeated with violet light (λ = 415 $m\mu$). The same effects were obtained, but somewhat larger quantities of light had to be applied.

DISCUSSION

FRANCK (1951), irradiated coleoptiles with white light (150 à 300 lux for 5 or 10 minutes) and placed the plants on the clinostat after they had been stimulated geotropically. He, too, observed that the geotropic curvature was maximally decreased and was sometimes even

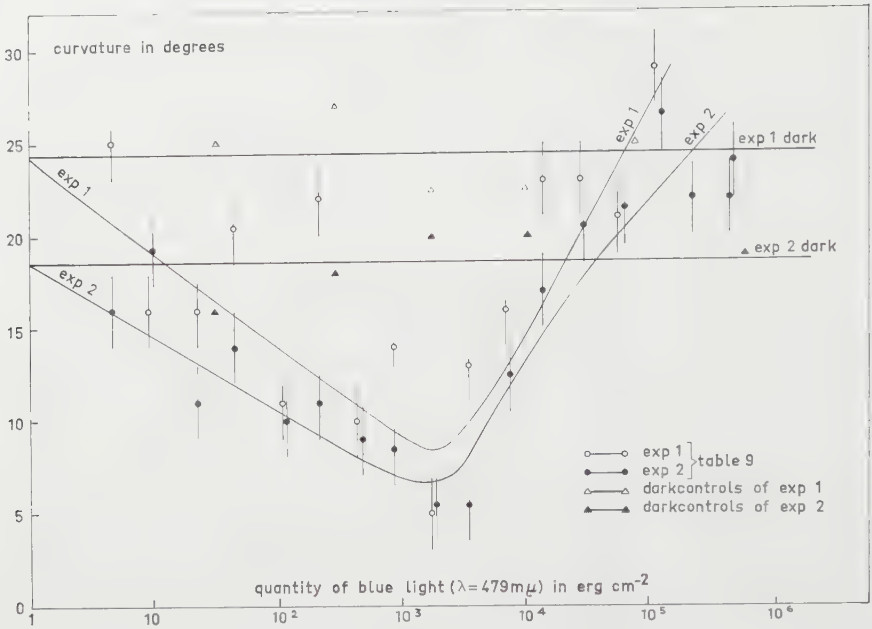


Fig. 5.

negative, when the interval between irradiation and geotropic stimulation amounted to half an hour. The growth of the curving coleoptiles was maximally enhanced under the conditions described.

From experiments yet to be described it will be seen, that these results can possibly be explained by comparing Franck's "white light" with a mixture of blue and red light.

CHAPTER V

COMBINATION OF RED AND BLUE IRRADIATION FROM DIRECTLY ABOVE

The next item to be studied was the influence of combined irradiations with blue and red light on the geotropic reaction of coleoptiles. By doing so it would be possible to determine whether blue and red light affect the geotropic reaction via the same mechanism, the former diminishing, the latter enhancing the geotropic reactions. In that case it should be possible to annihilate the influence of blue light by an irradiation with red or far red light and vice versa. According to Blaauw-Jansen (1959), the perception of red light is localized in the primary leaf. This statement can account for the fact that the influence of red light on the coleoptile is not effectuated until 30 minutes have passed, whereas blue light is believed to affect the coleoptile itself and therefore acting immediately.

In chapter XI it will be demonstrated that the statement on the role of the primary leaf is untenable, but this is of no consequence here.

Table 11 shows the working scheme together with the results of an experiment, satisfying the demands stated in the beginning of this chapter.

TABLE 11

1st irradiation	1st lapse (dark) in min	2nd irradiation	2nd lapse (dark) in min	curvature in degrees ("Exposure time" = 60 min)
red (7000 erg cm^{-2})	30	blue (2700 erg cm^{-2})	0	20
none	—	none	—	10
red (7000 erg cm^{-2})	30	none	—	22
none	—	none	—	15.5
red (7000 erg cm^{-2})	30	blue (2700 erg cm^{-2})	0	21
none	—	none	—	12
red (7000 erg cm^{-2})	30	none	—	23.5
none	—	none	—	16
blue (2700 erg cm^{-2})	30	none	—	7
none	—	none	—	16
blue (2700 erg cm^{-2})	0	red (7000 erg cm^{-2})	30	6
none	—	none	—	17
blue (2700 erg cm^{-2})	0	red (7000 erg cm^{-2})	30	5
none	—	none	—	16

From the values given in this table it is clear that the influence of red light is not annihilated by an irradiation with blue light nor vice versa. The experiment of table 11 was completed by the next experiments (tables 12 and 13) in which a red irradiation was immediately followed by an irradiation with blue light.

TABLE 12

1st irradiation	1st lapse (dark) in min	2nd irradiation	2nd lapse (dark) in min	Curvature in degrees ("Exposure time" = 60 min)
red (7000 erg cm^{-2})	30	none	—	26
none	—	none	—	13
red (7000 erg cm^{-2})	30	blue (2700 erg cm^{-2})	0	27
none	—	none	—	15
red (7000 erg cm^{-2})	0	blue (2700 erg cm^{-2})	30	5.5
none	—	none	—	18.5
none	—	blue (2700 erg cm^{-2})	30	4.5
none	—	none	—	18

The results of these three experiments were confusing, as it seemed that

a) a red irradiation immediately followed by a blue one, or vice versa, always produced the same effect as if there had only been an irradiation with blue light.

b) a red or blue irradiation, followed, after 30 minutes, by an

TABLE 13

1st irradiation	1st lapse (dark) in min	2nd irradiation	2nd lapse (dark) in min	Curvature in degrees ("Exposure time" = 60 min)
red (7000 erg cm^{-2})	30	none	—	21.5
none	—	none	—	18
red (7000 erg cm^{-2})	30	blue (2700 erg cm^{-2})	30	10
none	—	none	—	16
red (7000 erg cm^{-2})	0	blue (2700 erg cm^{-2})	30	7
none	—	none	—	16
blue (2700 erg cm^{-2})	30	none	—	4.5
none	—	none	—	20
blue (2700 erg cm^{-2})	30	red (7000 erg cm^{-2})	0	9
none	—	none	—	19
blue (2700 erg cm^{-2})	30	red (7000 erg cm^{-2})	30	13.5
none	—	none	—	17
blue (2700 erg cm^{-2})	0	red (7000 erg cm^{-2})	30	9.5
none	—	none	—	21

irradiation with blue or red light resp. only showed the effect of the first irradiation.

c) when in case b) a second lapse is inserted (i.e. the interval of time between the beginning of the second irradiation and the moment of putting the plants in a horizontal position) always an effect is produced as if there had only been an irradiation with blue light (cf. a).

DISCUSSION

The effect of combined irradiations is incomprehensible at this stage of the investigation. The reader is invited to dismiss the problem till more data will be placed at his disposal.

SUMMARY

("red" means: 7000 erg cm^{-2} of 660 mμ; "blue" means: 2700 erg cm^{-2} of 479 mμ)

a) "red" —lapse of 0 min.—"blue"—lapse of 30 min.
or
"blue"—lapse of 0 min.—"red" —lapse of 30 min.
→ decreased geotropic reaction (the same effect as from "blue" only)

b) "red" —lapse of 30 min.—"blue"—lapse of 0 min. → increased geotropic reaction (the same effect as from "red" only)

"blue"—lapse of 30 min.—"red" —lapse of 0 min. → decreased geotropic re action (the same effect as from "blue" only)

c) "red" —lapse of 30 min.—"blue"—lapse of 30 min.
or
"blue"—lapse of 30 min.—"red" —lapse of 30 min.
→ decreased geotropic reaction (the same effect as from "blue" only, but less pronounced).

CHAPTER VI

FURTHER INVESTIGATION OF THE INTERACTION OF BLUE, RED AND FAR RED LIGHT IN THE GEOTROPIC REACTION

To disentangle the complications risen in Chapter V it was thought necessary to subject the coleoptiles to a *series of quantities* of blue light (as used in the experiments of chapter IV), immediately, or after a lapse of 30 minutes, preceded by an irradiation with red light.

Firstly in chapter V was found

"red"—lapse of 0 min—"blue"—lapse of 30 min $\rightarrow$ the same effect as that of blue light only, viz. decreased geotropic reaction,

and so it was thought necessary to investigate whether this finding also holds when, instead of 2700 erg cm^{-2} of blue light, other quantities are applied.

Table 14 and Fig. 6 and 7 show working scheme and results of this part of the investigation. (This working scheme will be referred to as "*scheme a*").

It is clear that irradiation with red light enhances the geotropic curvature of the coleoptiles for most quantities of blue light. Thus, under the conditions described, the effects of blue and of red light are additive, at least for the lower quantities of blue light.

TABLE 14

Quantity of blue light in erg cm^{-2}	Curvature in degrees ("exposure time" = 60 min)							
	after irradiation with blue light only		after irradiation with blue light, immediately preceded by 7000 erg cm^{-2} of red light		after irradiation with 7000 erg cm^{-2} of red light only		of dark controls	
	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2
					21	24.5	20	16.5
2	18	17	23	25.5				
9	21	16; 17.5; 15; 17.5	24	24; 23.5; 24; 24.5				
45	14		21					
225	12		10					
900	7		11					
					22	26.5	16	20.5
3,600	9	4.5	13	8				
14,000	9		13					
60,000	19		19					
230,000	20	15; 15.5	17	18.5; 18				
					21	27.5	17	18.5
460,000	18		20					
						22.5	17	19.5
680,000		19; 19.5		18.5; 18.5				

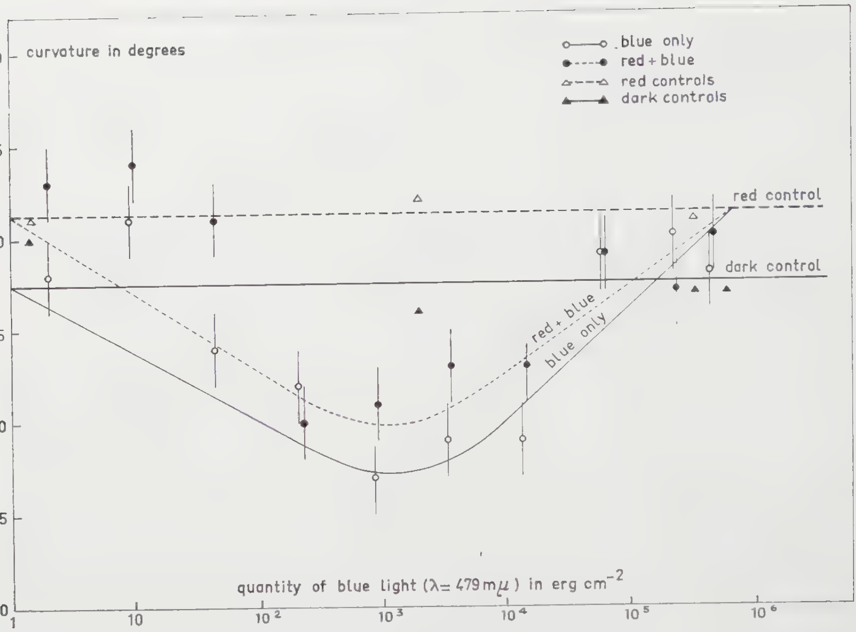

curvature in degrees
blue only
red + blue
red controls
dark controls
red control
dark control
red + blue
blue only
quantity of blue light (λ= 479 mμ) in erg cm-2
30
25
20
15
10
5
0
1
10
10 2
10 3
10 4
10 5
10 6

Fig. 6.

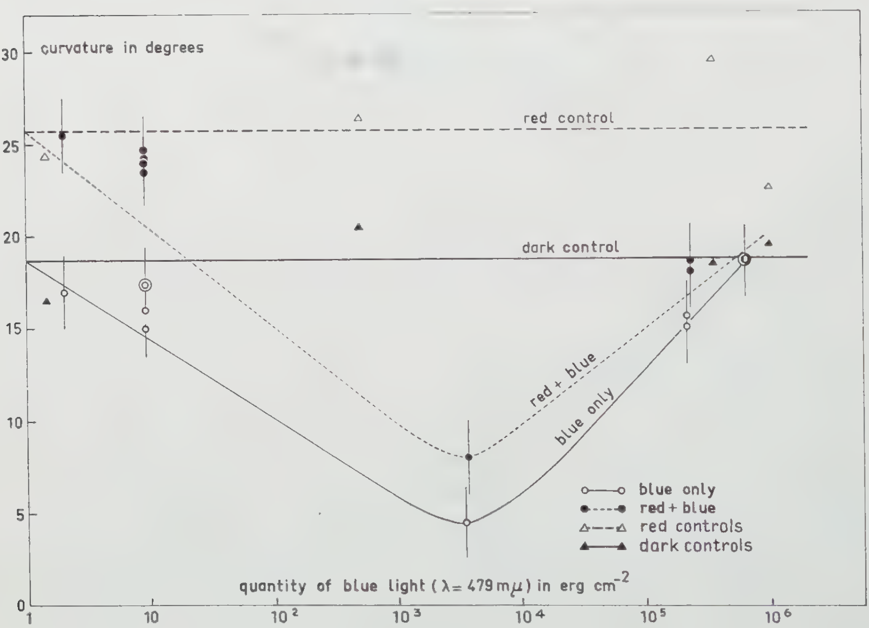

curvature in degrees
red control
dark control
red + blue
blue only
blue only
red + blue
red controls
dark controls
quantity of blue light (λ= 479 mμ) in erg cm-2
30
25
20
15
10
5
0
1
10
10 2
10 3
10 4
10 5
10 6

Fig. 7.

Secondly in Chapter V it was stated that
"red"—lapse of 30 min—"blue"—lapse of 0 min → the same effect as that of "red" only, viz. increased geotropic reaction,
and this statement was further examined too.

In this case a difficulty arose, because it is impossible to keep the lapse between red and blue illumination and the lapse between red illumination and the moment of placing the plants horizontal, both equal to 30 minutes, because of the fact that the administration of larger quantities of blue light required up to 15 minutes.

By way of compromise a working scheme was chosen in which the lapse between red irradiation (7000 erg cm^{-2}) and the moment of placing the plants horizontal, was fixed at 50 minutes, and the moment of the beginning of the blue irradiation was chosen in such a way that the plants had to be placed horizontal, immediately after the end of the blue irradiation, so the lapse between red and blue irradiation varied from 50 to 35 minutes.

In this way table 15 and Fig. 8 originated (*scheme b*).

Table 15 and Fig. 8 indicate that neither red + blue nor blue light only had a pronounced effect on the geotropic behaviour of the coleoptiles, possibly with the exception of an increase of the curvature in the range of the high quantities of blue light.

Hence it follows that the influence of red light on the coleoptile in the range of lower quantities of blue light, is independent of the influence of blue light. The experiment according to *scheme b* seems

TABLE 15

Quantity of blue light in erg cm^{-2}	Curvature in degrees ("exposure time" = 60 min)			
	after irradiation with blue light only	after irradiation with 7000 erg cm^{-2} of red light, followed after 50–35 min by blue light	after irradiation with 7000 erg cm^{-2} of red light only	of dark controls
			22	20
			20	18
2	18	—		
9	16	20		
45	17	—		
225	20	18		
900	19	20		
3,600	23	22		
14,000	24	23		
54,000	23	18		
230,000	29	24		
460,000	20	26		
			20	21
				19

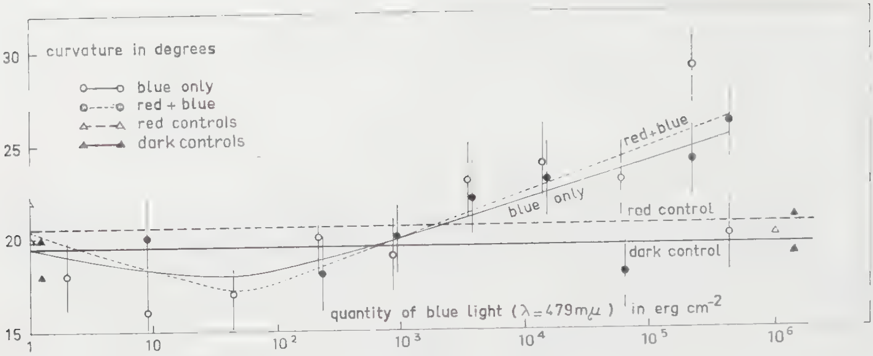


Fig. 8.

not to be conclusive with respect to the interplay of red and blue light because of the unfavourable intervals of time between irradiation and geotropic exposure. Supposing that the coleoptile requires an interval of time of 30 minutes in the vertical position to response on the irradiation either of red or blue light, then *scheme a* meets this requirement for both wave lengths, but in *scheme b* there is not enough time for the coleoptile to respond on blue light and the "waiting time" is too long for red light.

This supposition was tested in the experiment of table 16.

Apparently the effects of red and blue light by themselves, observable when applying a "waiting time" of 30 minutes, have disappeared when this interval is 50 minutes or more (table 16c and h). The blue

TABLE 16

A	1st irradiation	1st lapse in min	2nd irradiation	2nd lapse in min	Curvature (2 series) in degrees ("Exposure time" = 60 min)	
a	none	—	none	—	20	25
b	red 7000 erg cm^{-2}	30	none	—	28	31
c	id.	50	none	—	21	21
d	id.	28	blue 2700 erg cm^{-2}	0	25	26
e	id.	48	id.	0	25	23
f	id.	48	id.	30	18	18
g	none	—	id.	30	8	12
h	none	—	id.	60	20	22
B						
a	none	—	none	—	20	25
b	red 7000 erg cm^{-2}	30	none	—	28	31
c	id.	50	none	—	21	21
d	id.	28	blue 2700 erg cm^{-2}	0	24	29
e	id.	48	id.	0	20	24
f	id.	48	id.	30	16	22
g	none	—	id.	30	13	14

light has no effect at all, when the "waiting time" is zero (d and e). It seems strange, however, that in case f (in A as well as in B) red light diminishes the effect of blue light after a lapse of 48 minutes (compare g).

In table 17 and Fig. 9 the results are presented of an experiment in which the plants were irradiated with blue light or red light only. The "waiting time" was varied.

TABLE 17

Irradiation	"waiting time" in min.	Curvature in degrees ("Exposure time" = 60 min)
red 7000 erg cm^{-2}	0	18; 22
id.	30	28
id.	60	16; 19; 19; 18
blue 2700 erg cm^{-2}	0	20
id.	30	5
id.	60	24; 18; 23
none	—	23; 23

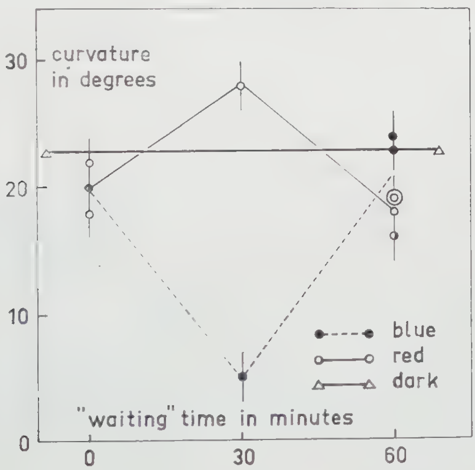


Fig. 9.

It will be noted that only when applying a "waiting time" of about 30 minutes an effect of the irradiation is observed.

Finally the experimental procedure given in *scheme a* was repeated with far red light.

"far red"—lapse of 0 min—"blue"—lapse of 30 min—"curvation time" 1 h.

Table 18, and Fig. 10 and 11 point out, that the results of this design are fundamentally comparable with those of the experiments in which the coleoptiles received a first irradiation with red light as was shown in Fig. 5 and 6.

TABLE 18

Quantity of blue light (λ = 479 mμ) in erg cm^{-2}	Curvature in degrees ("Exposure time" = 60 minutes)							
	after irradiation with blue light only		after irradiation with blue light, immediately preceded by 20,000 erg cm^{-2} of far red light		after irradiation with 20,000 erg cm^{-2} of far red light only		of dark controls	
	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2	exp. 1	exp. 2
none					19	20	15	16
2	15	19	26	23				
9	17	16	21	19				
none							17	17
45	15.5	15	19	17				
225	6	6	9.5	12				
900	5.5	9	8	11				
none					23	20	19.5	17
3,600	6.5	4	8.5	10				
13,500	9	12	6.5	9				
none							20	18
60,000	12	12	11	14				
230,000	—	19	23	18				
none					23.5	24	17	18
460,000	19	25	16,5	23				

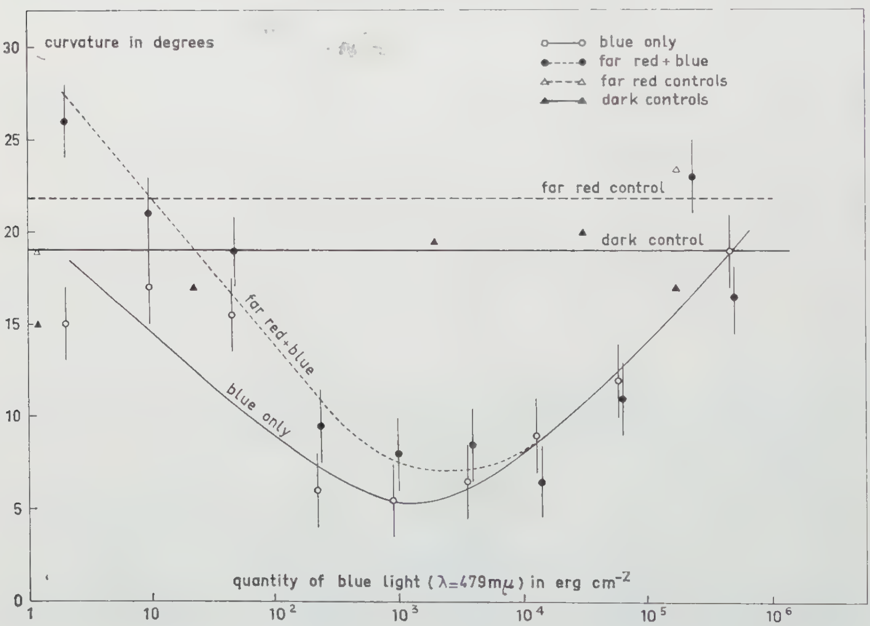


Fig. 10.

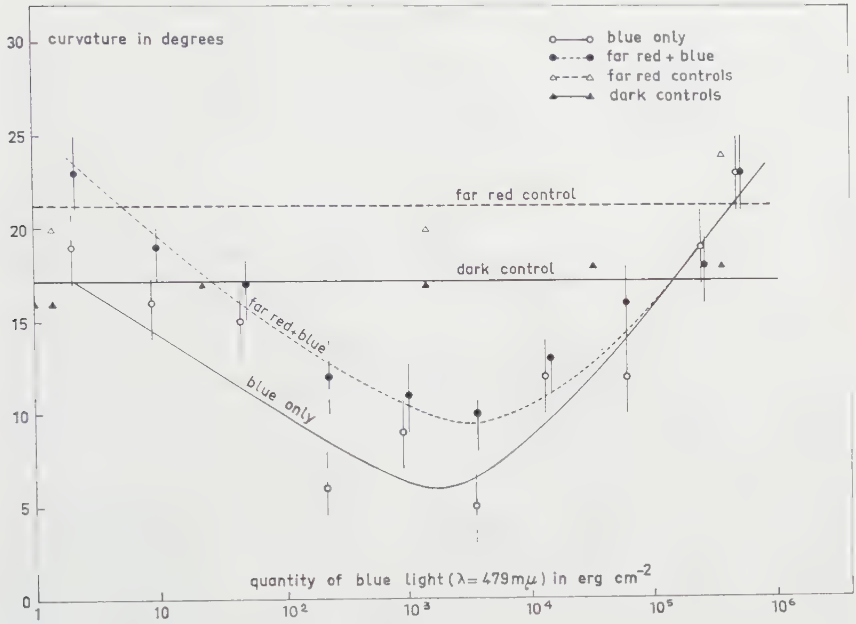


Fig. 11.

Discussion

In the preliminary experiments, mentioned in chapter III, the optimal "waiting time" for the effects of red and far red light was found to be 30 minutes.

Although in the experiments of chapter IV, on the influence of blue light, the same "waiting time" of 30 minutes was maintained, analogous to the design of the experiments of chapter III, this principle had to be dropped in chapter V in which a "waiting time" of 30 minutes could not always be applied in combination with the blue irradiations. Till then, the influence of blue light on the coleoptile was considered to be effectuated directly, and the "waiting time", therefore, to be of no importance.

This led to embarrassing results. It will be noted that, in the discussion of chapter IV, the paper of Franck (1951) was mentioned, who considered a "waiting time" of 30 minutes to be optimal for obtaining maximal effect of white light on geotropic reaction. Unfortunately, we came across his paper not before the experiments, described thus far, were finished. Our conclusion that not only red light but also blue light requires a period of about 30 minutes to influence the geotropic reaction of the coleoptile confirms his statement about the influence of white light.

As we have no data yet on which to advance any hypothesis about the mechanism of the effect of light on the gravitational response, a discussion on this mechanism has to be postponed till chapter XII.

SUMMARY OF CHAPTER VI

It is demonstrated that the geotropic reaction of the coleoptile is influenced by red, far red, and blue light, independent of one another and that a period of about 30 minutes has to be inserted between the beginning of the irradiation and the beginning of the geotropic exposure for blue as well as for red and far red light, to obtain any effect.

CHAPTER VII

THE CAUSE OF THE LIGHT-INDUCED SUPPRESSION AND ENHANCEMENT OF THE GEOTROPIC REACTION

We wanted to get information about the way in which the enhancement and the suppression of the geotropic reaction is effected; an enhancement can be brought about either by an increase of the growth rate of the convex side of the coleoptile or by a decrease of the growth rate of the concave side. Suppression of the geotropic curvature can be caused by any of the two reverse processes. In addition, knowledge of the influence of irradiation on the growth rate of coleoptiles placed vertical is indispensable for an explanation of the mechanism of geotropism.

For instance, if the suppression of the curvatures by blue light would appear to be a consequence of an enhancement of the growth rate of the concave side, it would make quite a difference for the interpretation of this phenomenon, whether this would be attended by an acceleration or by a retardation of the growth rate of a coleoptile orientated vertical.

Consequently a series of experiments was set up to investigate the growth rates of vertical and horizontal coleoptiles, after irradiation or in complete darkness. The method of investigation was described in chapter II. Shadowgraphs were made at intervals of 30 minutes during two hours on the usual Gevaert Document Rapid photographic paper by means of monochromatic green light (560 mμ).

In tables 19 and 20 two experiments are represented in which coleoptiles were allowed to curve geotropically with "waiting times" of 0, 30, and 60 minutes. The increases in length of their convex and concave sides are compared with the increase in length of vertical coleoptiles subjected to the same irradiation and in the same period after irradiation. The results are graphically recorded in Fig. 12 and 13.

Table 19 and Fig. 12 show:

a) that the decrease in curvature occurring after irradiation with 2700 erg cm^{-2} of blue light is due to the growth rate of the concave side of the coleoptile being higher than that of the dark control (Fig. 12*c*),

b) that the increase in curvature occurring after irradiation with 7000 erg cm^{-2} of red light is due to the growth rate of the convex side of the coleoptile being higher than that of the dark control (Fig. 12*f* and *i*),

TABLE 19

	Irradiation	"Waiting time" in min	Curvature in degrees 1)	Increase in length during 60 minutes in mm: Horizontal, Concave side 1)	Horizontal, Convex side 1)	Vertical 2)
a	none	—	17	0.05	0.51	0.60
b	blue (2700 erg cm^{-2})	0	15	0.08	0.49	0.58
c	id.	30	5	0.35	0.49	0.57
d	id.	60	15	0.15	0.56	0.36
e	red (7000 erg cm^{-2})	0	16	0.09	0.52	0.40
f	id.	30	22	0.08	0.72	0.55
g	none	—	17	0.07	0.55	0.41
h	red (7000 erg cm^{-2})	0	15	0.06	0.49	0.36
i	id.	30	23.5	0.00	0.66	0.57
j	id.	60	14	0.19	0.58	0.61

TABLE 20

a	none	—	15.5	0.18	0.61	0.56
b	far red (20,000 erg cm^{-2})	0	16.5	0.14	0.60	0.48
c	id.	30	22.5	0.11	0.73	0.70
d	id.	60	18.5	0.29	0.71	0.75
e	blue (400,000 erg cm^{-2})	0	27	0.26	1.02	0.69
f	id.	30	18	0.48	0.98	0.87
g	id.	60	16	0.23	0.67	0.57

1) Each figure giving the mean of 25 plants approximately (3 trays).
2) Each figure giving the mean of 16 plants approximately (2 trays).

c) that in two cases, where the curvature is neither increased nor decreased, the straight growth is much lower than that of the dark control (Fig. 12*d*, *e*),

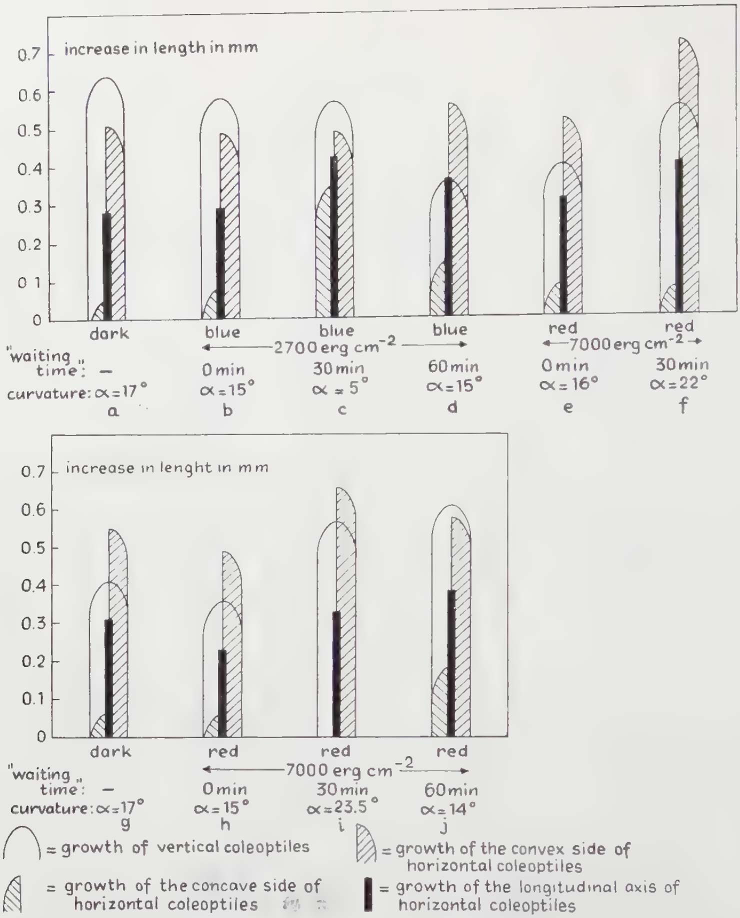
d) that the non-influenced curvature mentioned in table 20, line 5 (7000 erg cm^{-2} of red light; "waiting time" = 60 minutes) may be attended by the growth rate of both the convex and the concave side of the coleoptile being higher than that of the dark control (Fig. 12*j*).

In the same way table 20 and Fig. 13 show:

a) that the increase in curvature occurring after irradiation with 20,000 erg cm^{-2} of far red light or with 400,000 erg cm^{-2} of blue light is due to the growth rate of the convex side of the coleoptile being higher than that of the dark control (Fig. 13*c* and *e*),

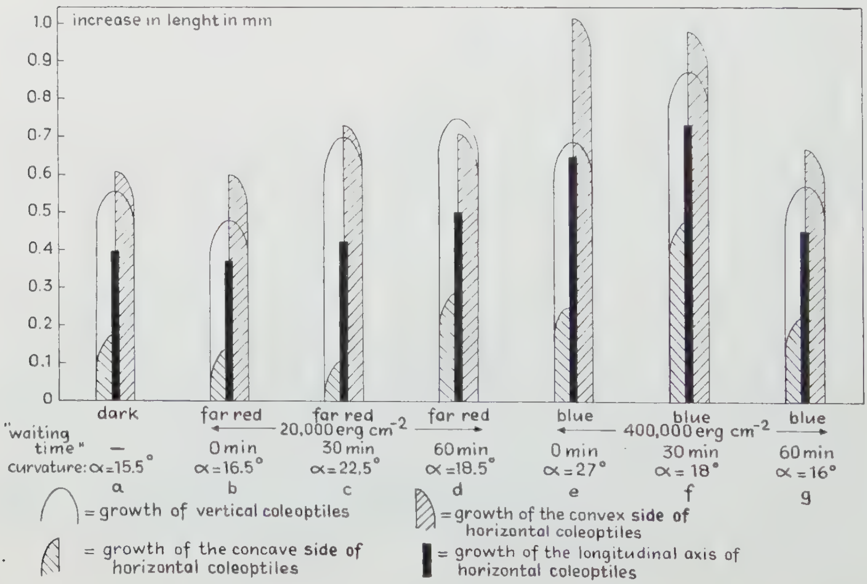
b) that the non-influenced curvature mentioned in table 20, line 6 (400,000 erg cm^{-2} of blue light; "waiting time" = 30 minutes) is attended by the high growth rate of both the convex and the concave side of the coleoptile (Fig. 13*f*),

c) that in the same case a straight growth rate much higher than that of the dark control is to be observed (Fig. 13*f*).



increase in length in mm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
dark
blue
blue
blue
red
red
2700 erg cm^{-2}
7000 erg cm^{-2}
"waiting time": —
0 min
30 min
60 min
0 min
30 min
curvature: α=17°
α=15°
α≈5°
α=15°
α=16°
α=22°
a b c d e f
increase in lenght in mm
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
dark
red
red
red
7000 erg cm^{-2}
"waiting time": —
0 min
30 min
60 min
curvature: α=17°
α=15°
α=23.5°
α=14°
g h i j
= growth of vertical coleoptiles
= growth of the convex side of horizontal coleoptiles
= growth of the concave side of horizontal coleoptiles
= growth of the longitudinal axis of horizontal coleoptiles

Fig. 12.



increase in lenght in mm
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
dark
far red
far red
far red
blue
blue
blue
20,000 erg cm^{-2}
400,000 erg cm^{-2}
"waiting time" —
0 min
30 min
60 min
0 min
30 min
60 min
curvature: α=15.5°
α=16.5°
α=22,5°
α=18.5°
α=27°
α=18°
α=16°
a b c d e f g
= growth of vertical coleoptiles
= growth of the convex side of horizontal coleoptiles
= growth of the concave side of horizontal coleoptiles
= growth of the longitudinal axis of horizontal coleoptiles

Fig. 13.

Discussion

The following objections can be raised against the experimental design used in the experiments of tables 19 and 20:

a) In view of the following data, it seems plausible to suppose that the green monochromatic light used for shadowgraphing, influenced the growth rate of the coleoptiles (table 21). During the time of observation vertical coleoptiles were shadowgraphed 4 times, horizontal ones 2 times, and for this reason the growth rate of both may not be quite comparable. So as to minimize the influence of green light, the growth phenomena were reinvestigated while using more sensitive photographic material.

Table 21
Total increase in length of vertical plants after two hours (in mm)

Shadowgraphed	Irradiation with		Dark
	Red light	Blue light	
Every 30 min	1.11	0.93	1.10
At the beginning and at the end of the growth period only	1.43	0.95	1.38

b) While shadowgraphing every 30 minutes, rapid changes in growth rate escape the notice of the investigator. So in experiments yet to be described shadowgraphs were made every 15 minutes.

c) In chapter IV it was stated that quantities of blue light of about 1800–3600 erg cm^{-2} maximally influence the geotropic reaction of the coleoptile.

As is indicated clearly in table 17, however, this statement is only true when working with a "waiting time" of 30 minutes. Larger quantities of blue light enhance the geotropic curvature, provided that the "waiting time" is reduced to 0 minutes. So, a series of experiments should be set up in which the quantity of blue light, administered to the coleoptiles, as well as the waiting time should be varied (Chapter VIII).

SUMMARY

The experiments described in this chapter were set up to provide data which should enable us to correlate the influence of irradiation of vertical plants with response and reactions to irradiation of horizontal plants. These experiments clearly pointed out that the lowering of the geotropic reaction by blue light is caused by an enhancement of the growth rate of the concave side of the coleoptile, whereas the increase of the geotropic curvatures by red and far red light is due to an enhancement of the growth rate of the convex side.

In those cases where no influence upon the curvature was observable, viz. irradiation with red light, combined with a waiting time of one hour or after irradiation with much blue light, combined with a "waiting time" of half an hour, it can be stated that the growth of both the convex and the concave side of the horizontal coleoptile is enhanced.

CHAPTER VIII

THE INFLUENCE OF THE "WAITING TIME" FOR VARIOUS QUANTITIES OF BLUE, RED AND FAR RED LIGHT ON THE GEOTROPIC REACTION

Blue light

Whereas, with a "waiting time" of 30 minutes, a quantity of blue light ($\lambda = 479$ mμ) of about 50,000 erg cm^{-2} does not influence the geotropic reaction of the coleoptile (cf. chapter IV) the geotropic reaction is markedly increased after irradiation with 400,000 erg cm^{-2}, applying a "waiting time" of 0 minutes (chapter VII).

This suggests that for influencing the geotropic reaction, discrete combinations of quantities of light and "waiting times" are required.

Two series of experiments were set up to settle this point: Firstly a series to study the influence of a number of "waiting times" for a number of fixed quantities of blue light. Secondly a series to study the influence of a number of quantities of blue light for a number of fixed "waiting times". This second series is meant to check the most salient data obtained in the first series.

The results of the first series of experiments are given in Fig. 14 *a-k*, and those of the second series in Fig. 15 *a-d* and Fig. 5, in which the "waiting times" are 0, 60, 75, 75, and 30 minutes resp. Each point represents the mean of two trays of c. 12 plants each.

Fig. 16 shows a spatial graph, schematically combining the Fig. 14 *a-k*, 15 *a-d* and 5. The most striking features in this graph are:

1) the reaction to irradiation with 1800–7200 erg cm^{-2} (a sharp fall at a "waiting time" of 30 minutes; a sharp rise at a "waiting time" of 75 minutes).

2) the reaction to irradiation with 100,000–500,000 erg cm^{-2} (a sharp rise at a "waiting time" of 0 minutes and a less pronounced one at a "waiting time" of 75 minutes).

Red light (660 mμ)

Fig. 17*a* and *b* and table 6 show that the relation between geotropic reaction, quantity of red light and "waiting time" is much less complicated than in the case of blue light.

Irradiations with 2800, 7000 or 90,000 erg cm^{-2} all show the same picture: when choosing a "waiting time" of 30 minutes an increase in curvature is noted.

Far red light (735 mμ)

As can be seen from Fig. 18 and from table 6 giving the results of experiments with 1,000,000 and 12,000 erg cm^{-2} resp. the response evoked by far red is essentially the same as that obtained with red light.

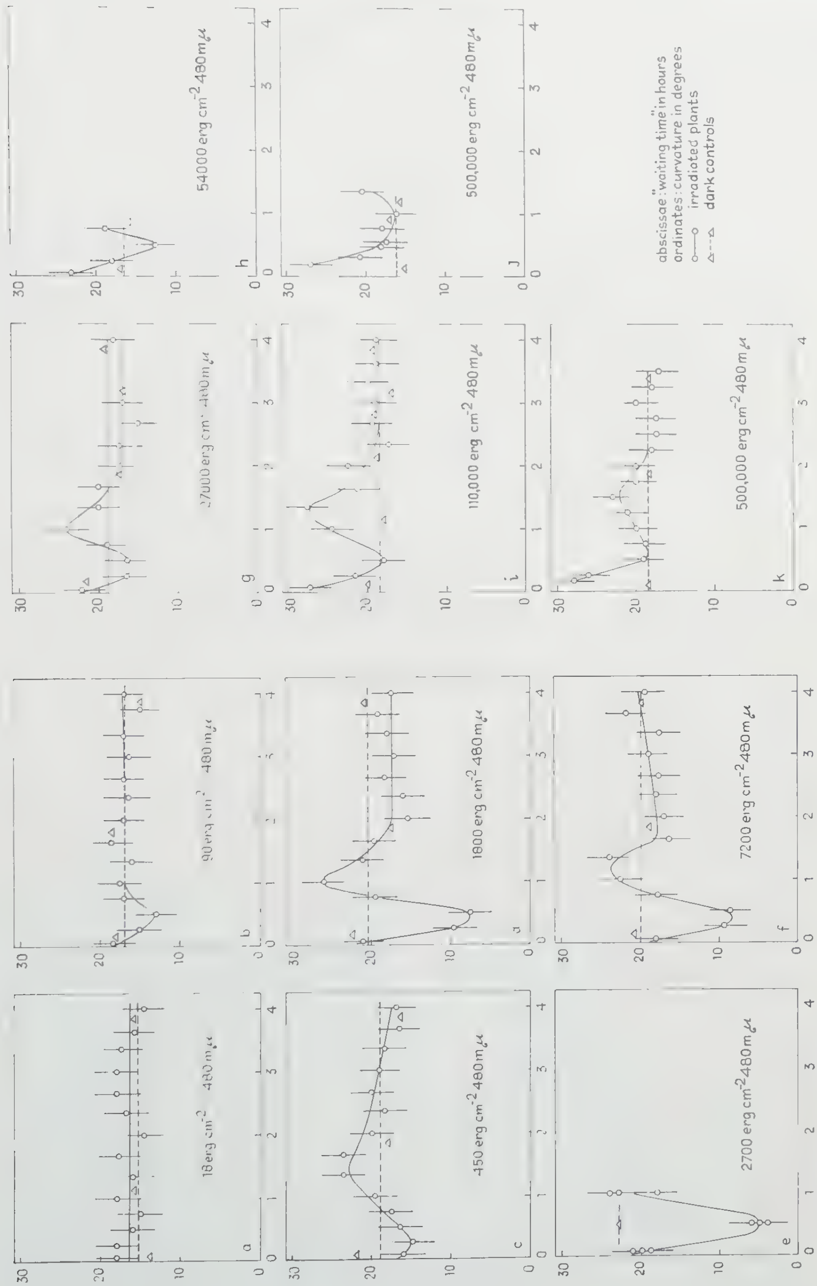

54000 erg cm-2 480 mμ
1800 erg cm-2 480 mμ
7200 erg cm-2 480 mμ
450 erg cm-2 480 mμ
2700 erg cm-2 480 mμ
110,000 erg cm-2 480 mμ
500,000 erg cm-2 480 mμ
500,000 erg cm-2 480 mμ
abscissae: "waiting time" in hours
ordinates: curvature in degrees
irradiated plants
dark controls

Fig. 14.

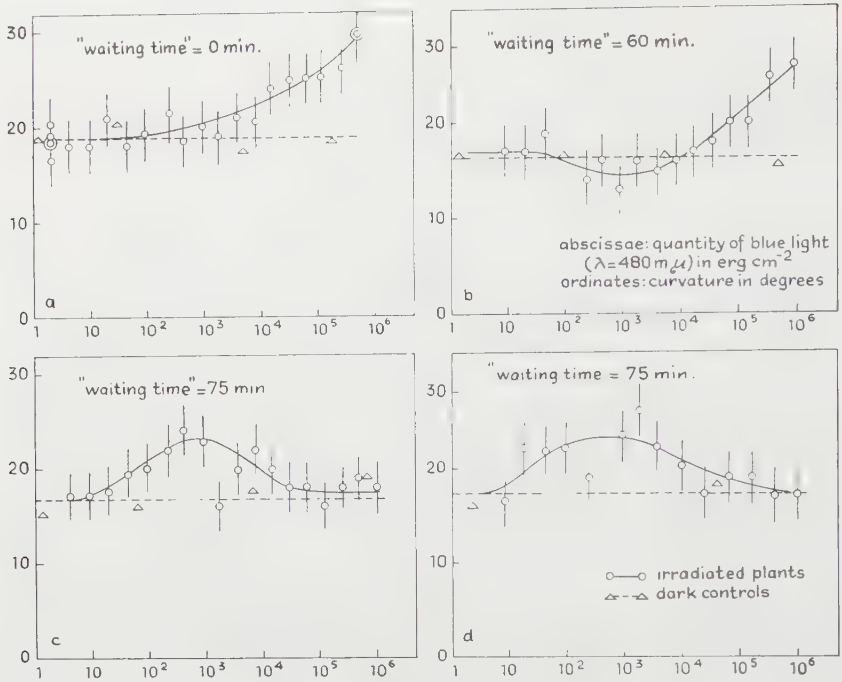

"waiting time" = 0 min.
a
"waiting time" = 60 min.
abscissae: quantity of blue light (λ=480 mμ) in erg cm⁻²
ordinates: curvature in degrees
b
"waiting time" = 75 min
c
"waiting time = 75 min.
irradiated plants
dark controls
d
0
10
20
30
1
10
10²
10³
10⁴
10⁵
10⁶

Fig. 15.

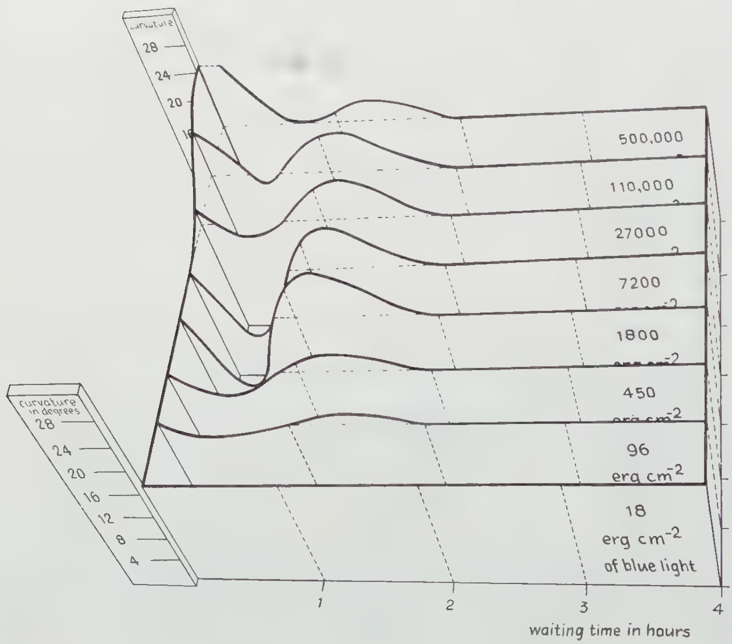

curvature in degrees
500,000
110,000
27000
7200
1800
450
96 erg cm⁻²
18 erg cm⁻² of blue light
waiting time in hours
1
2
3
4

Fig. 16.

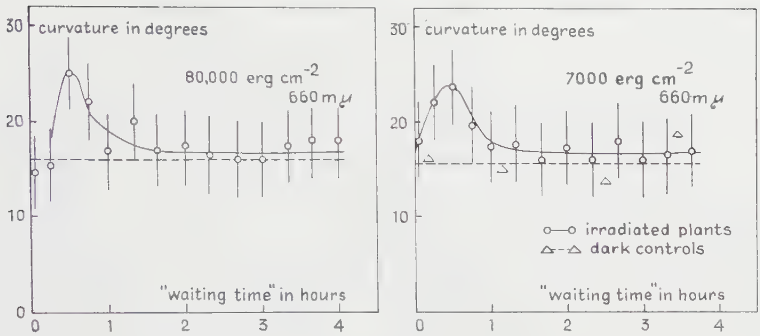


Fig. 17.

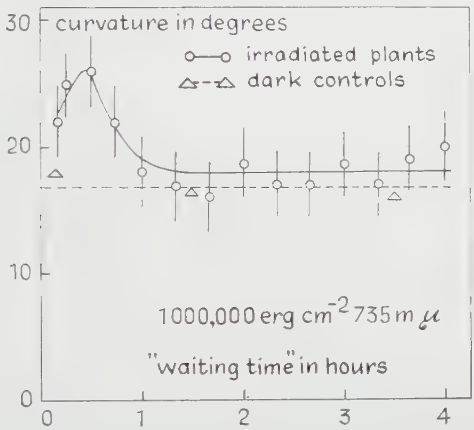


Fig. 18.

Discussion

As to the relation between quantity of light and "waiting time", Franck (1951) found a "waiting time" of 30 min to be necessary for a maximal geotropic reaction when administering a quantity of 150 lux of white light during 5 minutes, and a "waiting time" of 20 minutes when giving 300 lux during 5 minutes.

A shift of that kind cannot be found in our experiments. Also, the rise and fall at "waiting times" shorter than 5 minutes mentioned by Franck lie outside our scope, as our shorter "waiting times" were 0 and 15 minutes.

The rise at a "waiting time" of 0 minutes found in experiments with large quantities of blue light may correspond with Franck's findings. Franck, however, manipulated his plants in red light, whereas the present author used very dim green light. Franck chose red light because it has no phototropic effect.

Blaauw-Jansen (1959) demonstrated that it has a tonic effect resulting in an enhancement of the phototropic reaction. Indeed,

experiments, described in this paper, show that this effect on the phototonus has a strong influence on the geotropic reaction of the coleoptile too. Franck's results are difficult to interpret because the influence of red light on the geotropic reaction only becomes evident when a "waiting time" of 30 minutes is applied, and because it is not known how much time elapsed between his manipulating the plants and the onset of the geotropic exposure.

We could demonstrate, however, that when the coleoptiles are irradiated with large quantities of blue light the influence of red light is less marked. Possibly Franck's experiments can be compared with our experiments about the influence of much blue light (Fig. 14*i*, *j*, *k*).

Presumably the curvature of Franck's dark control is stronger than the curvature of the dark control of our Fig. 14, because his plants were exposed to a large dose of red light. This may explain his finding of an enhancement of the geotropic curvature with a waiting time of a few minutes, of a reduction of the curvature till below the control values with a waiting time of 30 minutes and a recovery of the curvatures with "waiting times" up to one hour. The enhancement with longer "waiting times" caused by irradiation with lower quantities of blue light, was not reported by Franck. Perhaps irradiation with white light does not evoke this enhancement.

We should like to drop this matter for the present and to concentrate our attention on finding an explanation for our own data as will be attempted in the next chapter.

SUMMARY

The relation between quantity of blue light, "waiting time" and geotropic reaction is summarized in Fig. 16.

The same relation with respect to red and far red light is much simpler, the only effect being a stimulation of the geotropic reaction when applying "waiting times" of about 30 minutes.

CHAPTER IX

THE INFLUENCE OF PRE-IRRADIATION ON THE GROWTH OF SEEDLINGS IN VERTICAL POSITION

As we wanted to compare the effects of irradiation on the growth of the curving horizontal coleoptile with the effects on the growth of the vertical one, in the next experiment a series of coleoptiles was shadowgraphed every other 15 minutes after being irradiated with different quantities of blue, red or far red light, on Gevaert O 53 graphic ortho film which shows fairly high sensitiviy to light of 560 mμ.

The results of this experiment will be given as graphical records only of the mean increase in length of approximately 25 plants during each period of 15 minutes.

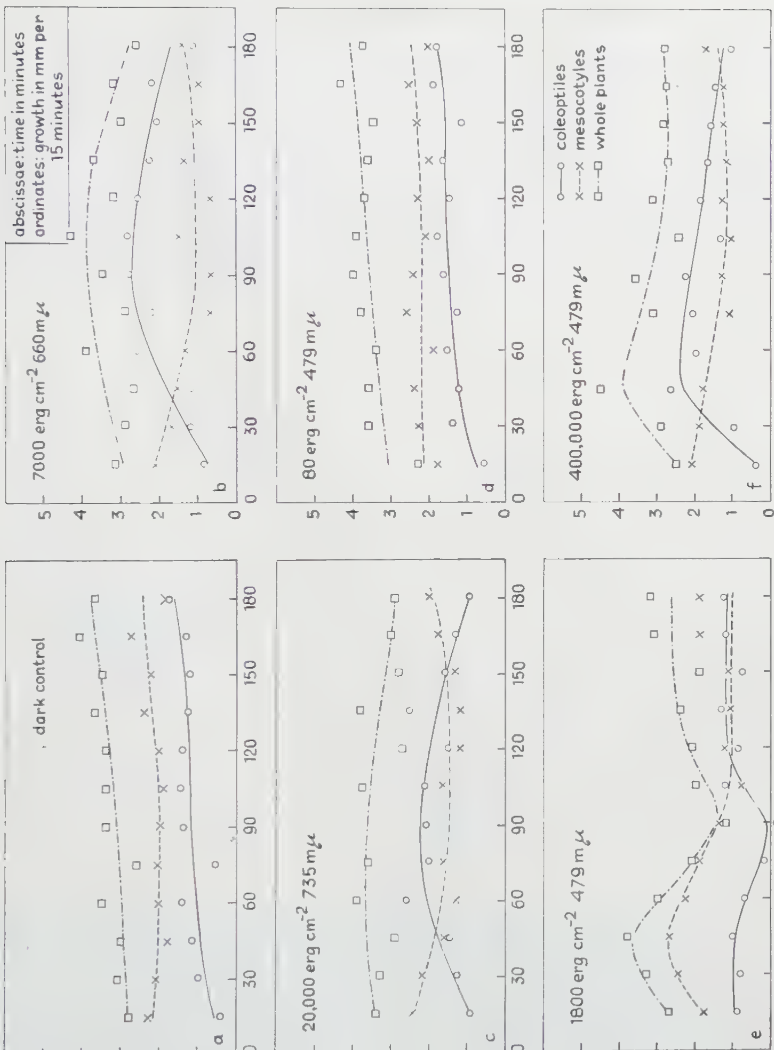


Fig. 19.

In Fig. 19*a-f* the increase in length is shown of "mesocotyls", "coleoptiles" and whole plants.[1])

These results were confirmed by a second experiment in which slightly different light quantities were administered to the plants and in which the growth measurements were continued during two hours

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[1]) For definitions see p. 402.

only. The records are shown in Fig. 20*a-d*. In this second experiment shadowgraphs were made with intervals of 10 minutes.

For the moment we shall only discuss the influence of irradiation on the growth of the coleoptile. The reactions of the mesocotyl will be considered in Chapter XII.

The growth of the whole plants is given for the sake of completeness. The graphs of Fig. 19 clearly indicate that 80 erg cm^{-2} of blue light does not influence the growth; 2700 erg cm^{-2} of blue light leads to a temporary reduction of the growth rate, lasting from the 45th till the 120th minute after irradiation, while 54,000 erg cm^{-2} (Fig. 20)

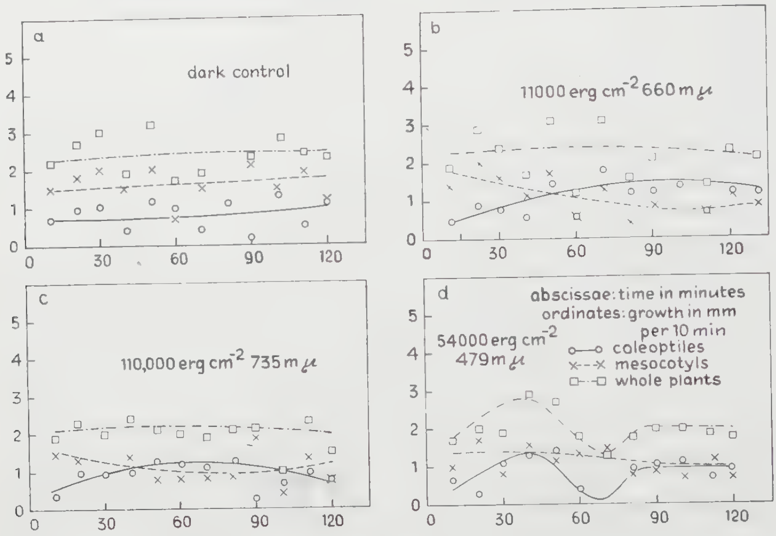

Fig. 20.

shows a rise and then a fall, and 400,000 erg cm^{-2} (Fig. 19) shows only a rise, immediately after the irradiation, that has vanished two hours later. Red and far red show essentially equal pictures: viz. a long lasting slight enhancement of the growth rate of the coleoptile.

These results were motive for Blaauw-Jansen (1962) to investigate the indole-3-acetic acid contents of coleoptiles at various points of time after irradiation with various quantities of blue light (see chapter XIV).

CHAPTER X

CORRELATION OF THE PHENOMENA OF GEOTROPISM AND PHOTOTROPISM

When we want to apply the prevailing views on the causes of phototropism to our findings on the influence of light on geotropism, it is necessary to correlate the various phenomena of phototropism

(first positive curvature, negative curvature, second positive curvature) with the suppression and enhancement of geotropic reactions by light.

A. Therefore, the following experiment was made: The experimental plants were divided over three groups, all of them were treated with a series of quantities of light of 479 mμ.

series a was irradiated from directly above.

series b was irradiated from two sides with the same quantities as *series a* with the aid of surface mirrors (Fig. 21). The narrow sides of the coleoptiles were irradiated.

series c was irradiated with the same quantities of light from one side by removing one of the lower mirrors (Fig. 21).

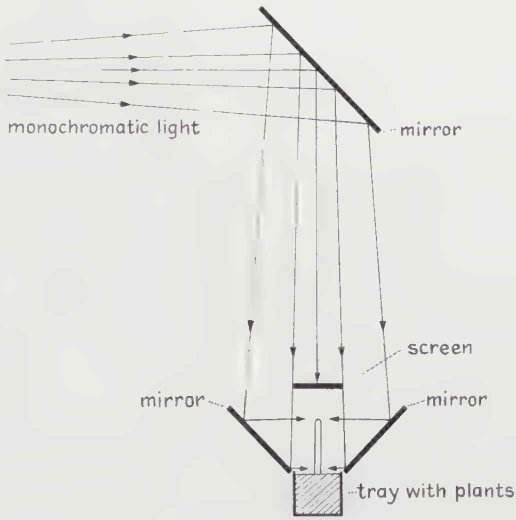

Fig. 21.

The plants of *series a* and *b* were placed horizontal after a "waiting time" of 30 minutes, the plants of *series c* were allowed to curve phototropically for 90 minutes, after which they were shadowgraphed.

To eliminate the influence of a possible difference in the intensity of the beams reflected by the two mirrors for *series b* a double number of trays was taken. In rapid succession two trays were irradiated with equal quantities of light. After a "waiting time" of 30 minutes one tray was turned on its left, the other on its right side.

In this way, two sets of figures were obtained, which were in close agreement with each other. In the 3rd column of table 22 the mean values of these two sets are given.

The results of the experiments mentioned under A are shown in table 22 and in Fig. 22.

It is clear from Fig. 22 that it makes no essential difference for the light quantity required to produce the minimum in the geotropic reaction whether the light is applied from above or from two sides.

TABLE 22

Quantity of light ($\lambda = 479$ mμ) in erg cm^{-2}	Geotropic curvature in degrees ("Exposure time" = 60 min)		Phototropic curvature in degrees (Developed within 90 min)
	light from above	light from 2 sides	
4		20	
16		18	
40	14		+20
80		12.5	
200	8	8	+25
400		5.5	
800	1	10	+12
1,600	13.5	7	+ 9
3,200	7	11.5	+ 1
6,500	7.5	12	— 9
13,000	9	13.5	—11.5
25,000	10.5	18	—10
50,000	20	19.5	— 7.5
100,000		20.5	
200,000	21	22.5	+ 5.5

dark controls: 19; 20; 20.5 and 21.5 degrees.

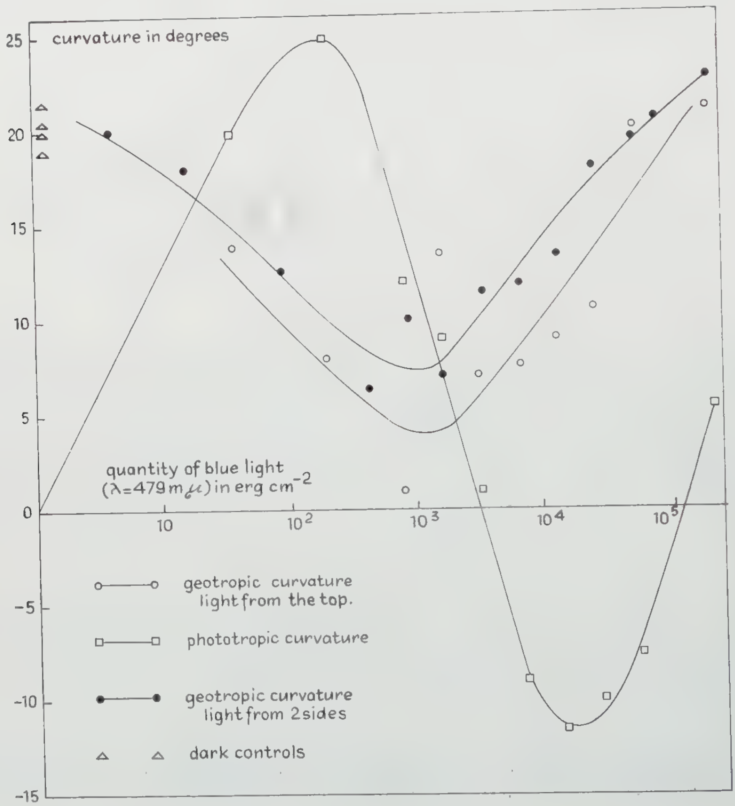

Fig. 22.

The negative phototropic reaction, however, occurs at much larger quantities of blue light than the minimum in the geotropic reaction. This feature may serve us in the general discussion of the phenomena described in this paper.

A further possible point of contact with the hypothesis proposed by Blaauw-Jansen (1959), explaining the phototropic behaviour of *Avena* coleoptiles, lies in the realm of the red - far red antagonism, as Blaauw-Jansen proved that the turning point from negative to second positive phototropic curvature shows red - far red antagonism.

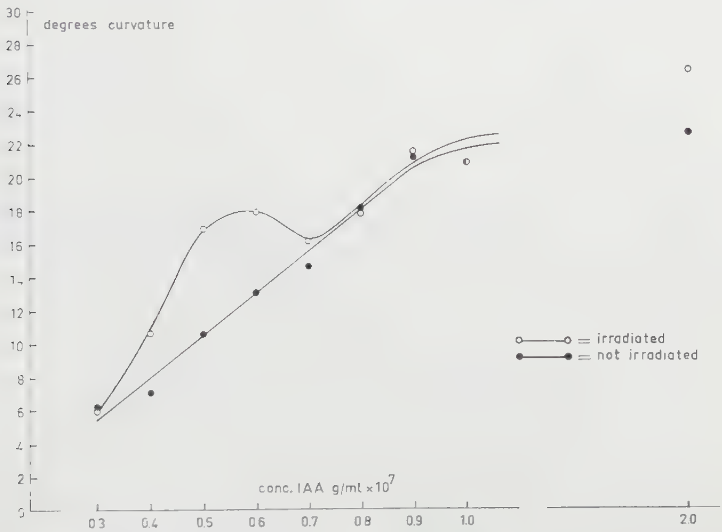

Fig. 23. (from Blaauw-Jansen, 1959)

At four instances in the experiments described in Chapters VII and IX we established an increased growth with plants that were pre-illuminated with blue light, viz.:

1. After pre-illumination with 2700 erg cm^{-2} a restoration, after an initial fall, in the growth curve of vertically placed coleoptiles (Fig. 19*e*) corresponding with the second rise in Fig. 20*d*,

2. After pre-illumination with 400,000 erg cm^{-2} a rise in the growth curve of vertically placed coleoptiles (Fig. 19*f*) corresponding with the first rise in Fig. 20*d*,

3. After pre-illumination with 400,000 erg cm^{-2} a rise in the growth of the convex side of the horizontal coleoptiles responsible for the enhancement of the geotropic curvature (Fig. 13*f*) under those conditions,

4. After pre-illumination with 2700 erg cm^{-2} an increase in the growth of the concave side of the horizontal coleoptile responsible for the lowering of the geotropic curvature under those conditions (Fig. 12 *c*).

If a red-far red antagonism could be demonstrated, either on the growth of vertically standing plants in the cases 1 or 2 or on the curvature in the cases 3 or 4, it would be probable that a point of contact with the phototropic behaviour had been hit upon.

Firstly the possibility mentioned under 1 was tested. The restoration of the growth rate appears about 90 minutes after the irradiation with blue light.

From our experiments with red light we know that the effect of red light appears only when the geotropic curvature is allowed to develop from the 30th to the 90th minute after irradiation. For this reason we decided to proceed according to the following working scheme:

time = 0: irradiation with blue ($\lambda = 479$ mμ) 1800 erg cm^{-2}
time = 45 min: irradiation with red ($\lambda = 660$ mμ) 7000 erg cm^{-2}
or: irradiation with far red ($\lambda = 735$ mμ) 20,000 erg cm^{-2}
or: both.

In this scheme the effect of blue light is expected to manifest itself from the 90th minute after the irradiation onward. The effect of red, far red or the combination of the two is anticipated to begin in the period between the 75th and the 135th minute.

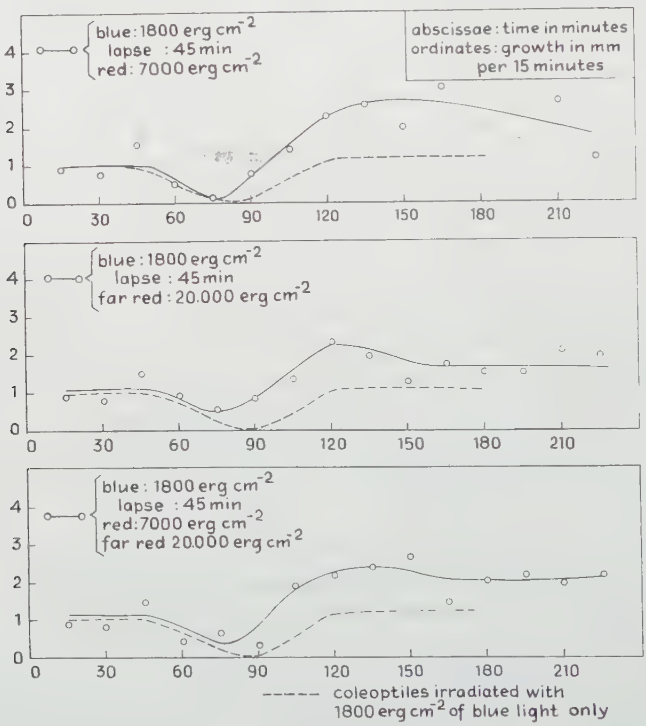

Fig. 24.

In the graphs of Fig. 24 the effect of these treatments on the growth of the coleoptile is given. Each point gives the mean of about 20 plants.

The broken line in each of the graphs is the coleoptile growth after the irradiation with blue light only (taken from Fig. 18*e*). From these data we conclude to the non-existence of a red - far red antagonism in this instance.

As to the second possibility, we see that the increased growth mentioned, appears immediately after the irradiation with blue light.

In the following working scheme the influences of blue and red or far red light are expected to occur at the same time too.

time = 0: red (7000 erg cm^{-2})
or: far red (20,000 erg cm^{-2})
or: both
lapse of 30 minutes
blue (400,000 erg cm^{-2}).

The effect of these treatments of the coleoptile growth is shown in Fig. 25.

Each point represents the mean of 20 coleoptiles.

The broken line in each of the graphs is the coleoptile growth after irradiation with 400,000 erg cm^{-2} of blue light only (taken from Fig. 19*f*). Neither in this case red-far red antagonism is apparent. Next, the influence of red and far red light on the suppression of the

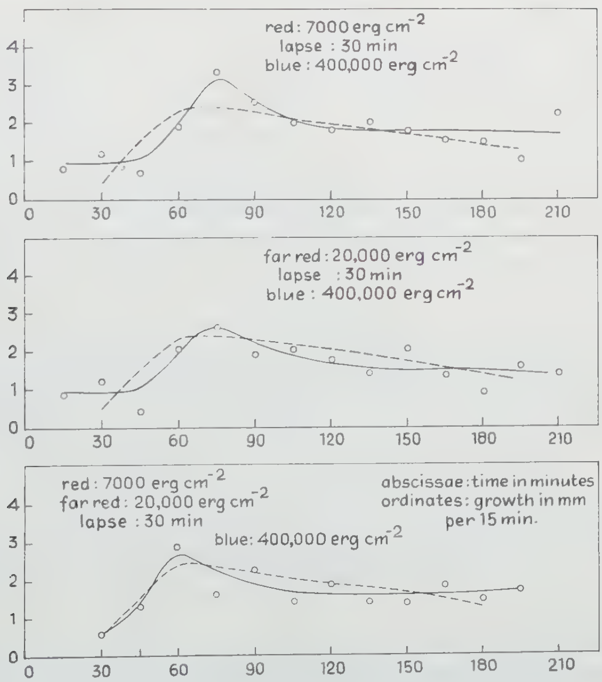

Fig. 25.

TABLE 23

Plants irradiated with			Curvatures in degrees ("Exposure time" =60 min)
blue	red	far red	
2700 erg cm^{-2}	—	—	5.5
2700 erg cm^{-2}	7000 erg cm^{-2}	—	7
2700 erg cm^{-2}	—	20,000 erg cm^{-2}	8.5
2700 erg cm^{-2}	7000 erg cm^{-2}	20,000 erg cm^{-2}	8
—	—	—	17

geotropic curvature by blue light was studied, according to 3. Table 23 shows that no red-far red antagonism was indicated by this experiment.

Likewise, as table 24 shows, the enhancement of the geotropic curvature after 400,000 erg cm^{-2} of blue light was not subject to the red-far red antagonism.

Working scheme:

time = 0: red or far red or both
lapse of 30 minutes
time = 30 min: 400,000 erg cm^{-2} of blue light (administered in the course of 9 minutes)
time = 39 min: plants put horizontal. First shadowgraph.
time = 99 min: second shadowgraph.

TABLE 24

Plants irradiated with			Curvature in degrees ("Exposure time" = 60 min)
red	far red	blue	
—	—	400,000 erg cm^{-2}	27
7000 erg cm^{-2}	—	400,000 erg cm^{-2}	31
—	20,000 erg cm^{-2}	400,000 erg cm^{-2}	31
7000 erg cm^{-2}	20,000 erg cm^{-2}	400,000 erg cm^{-2}	35
—	—	—	17

SUMMARY

The minima in the curves representing the geotropic reactions occur at much lower quantities of blue light than the negative phototropic curvature does.

An antagonistic effect of red and far red light, combined with irradiations with 2700 of 400,000 erg cm^{-2} or blue light could not be demonstrated.

CHAPTER XI

THE SITE OF PERCEPTION OF RED, FAR RED AND BLUE LIGHT

A. BLUE LIGHT

From the experiments in Chapter X with irradiation from two sides or from right above can be concluded that the percepting site (cell or group of cells) is more or less isodiametric.

B. Red, far red and blue light

For these light qualities the influence of the primary leaf on the perception was investigated. Indirect evidence exists (Blaauw-Jansen, 1959) for the possibility that the tip of the primary leaf is the site of perception of red and far red light.

It was thought useful to study the reactions of coleoptiles from which the primary leaf was excised. This was performed in the following way. With the device represented in Fig. 26 (a, b) the coleoptile was slit at two opposite sides, from about 5 mm. below the top to about 3 mm. over the node. The incisions were made as shown in Fig. 26 c.

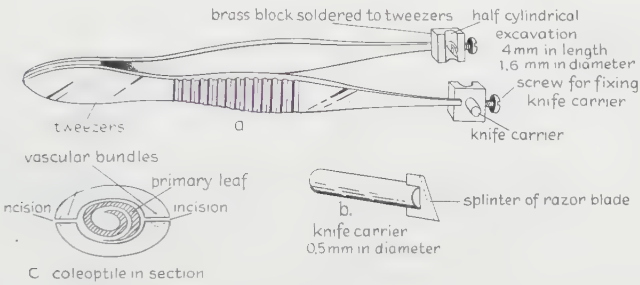

Fig. 26.

With the aid of a pair of tweezers with very thin, flattened points the primary leaf was removed completely from the coleoptile. It is feasible to perform these actions without bruising the coleoptile. It proved to be impossible for the author to remove the primary leaves in total darkness, but a light intensity of 300 erg cm^{-2} sec^{-1} of green light ($\lambda = 560$ mμ) was sufficient.

The relative humidity of the dark room was raised to 96 % in order to avoid the desiccation of the coleoptiles.

In every tray of 15 plants 5 coleoptiles were slit up, without removing the primary leaves, from 5 other coleoptiles the primary leaves were removed, the remaining 5 were left intact.

Ten minutes approximately were needed to treat a tray of plants in this way. Next, the trays were placed in darkness for about 2 hours and subsequently irradiated. After a "waiting time" of 30 minutes the plants were put horizontal and were shadowgraphed for the first time. A second shadowgraph was made after another hour.

The results of two experiments of this kind are given in table 25.

These experiments show, that slitting the coleoptile decreases the geotropic reaction of the plants but that removal of the primary leaf does not essentially influence the effect of irradiation whatever the light quality or quantity used.

Discussion

It is known that the sensitivity of the coleoptile for blue light is maximal in its tip. Lange (1928) stated that the maximal sensitivity

TABLE 25

	Curvature in degrees ("waiting time" = 30 min) ("exposure time" = 60 min)									
	dark		red 7000 erg cm^{-2}		far red 20,000 erg cm^{-2}		blue 1800 erg cm^{-2}		blue 400,00 erg cm	
exp. no.	1	2	1	2	1	2	1	2	1	2
intact plants	14.5	16.5	24	28	22	23	9.5	10	23	22
plants slit only	13	11	21	19.5	18	13.5	7	2	17.5	15
prim. leaf removed . .	13.5	11	23	19	19	16	4	3	18	17

for light is located in the uppermost 50 μ of the coleoptile, that is in the top layer of the five layers of small cells of which the tip of the coleoptile consists. The experiments presented in Fig. 22 show that the difference in the effects of low and high quantities of blue light cannot be caused by a difference in the place of perception, as for both of them the same effect is obtained, the irradiation being applied either from above or from two sides. Apparently both of them exercise their influence on the topmost cells.

We did exclude the primary leaf as the organ which perceives the red and far red light. Perception by the primary leaf and indirect influence of red light via the primary leaf on the coleoptile offered an explanation of the difficulty that red light influences the growth of the coleoptile but induces no phototropic curvature when applied unilaterally (BLAAUW-JANSEN, 1959).

We are not able to suggest any explanation for the influence of red and far red light, neither with respect to our own experiments, nor to Blaauw-Jansen's.

CHAPTER XII

SUPPLEMENTARY EXPERIMENTS

Two further investigations on one of the most salient results of our studies seemed opportune, viz.:

1) The way in which the geotropic curvature and the growth of the coleoptile develop in the course of the "exposure time" after irradiation with 2700 erg cm^{-2} of blue light ($\lambda = 479$ mμ) and applying "waiting times" of 0, 30, 60, 75 and 120 minutes.

2) The tonic condition of the coleoptile in the period beginning with the third hour after the irradiation with blue light, when the geotropic reaction is the same as that of the dark control.

So *firstly* we irradiated 10 trays, each containing c. 15 carefully selected plants, with 2700 erg cm^{-2} of blue light from directly above and put them on their sides in pairs after 0, 30, 60, 75 and 120 minutes resp. Each plant was provided with a grain of vermiculite as a mark

about 1 cm below the top. The plants were allowed to curve during a period of 75 minutes and were shadowgraphed on Gevaert O 53 graphic ortho film at the 0th, 15th, 30th, 45th, 60th, and 75th minute of this period.

The same was done with two trays with non irradiated plants. With the aid of a photographic enlarger supplied with a lens of high quality (Leitz Elmar 1:3.5) from these shadowgraphs perfectly sharp images, 10 times enlarged, were made and from these images the growth of the concave and the convex side was determined in the way described in chapter II, Fig. 2.

Finally in each of the shadowgraphs the curvatures of the plants from the 12 trays mentioned above were measured.

Two other trays were irradiated with the same quantity of blue light. The plants were allowed to grow in the vertical position and also shadowgraphed every 15 minutes during 3 hours.

In this case too the same was done with two trays with non-irradiated plants.

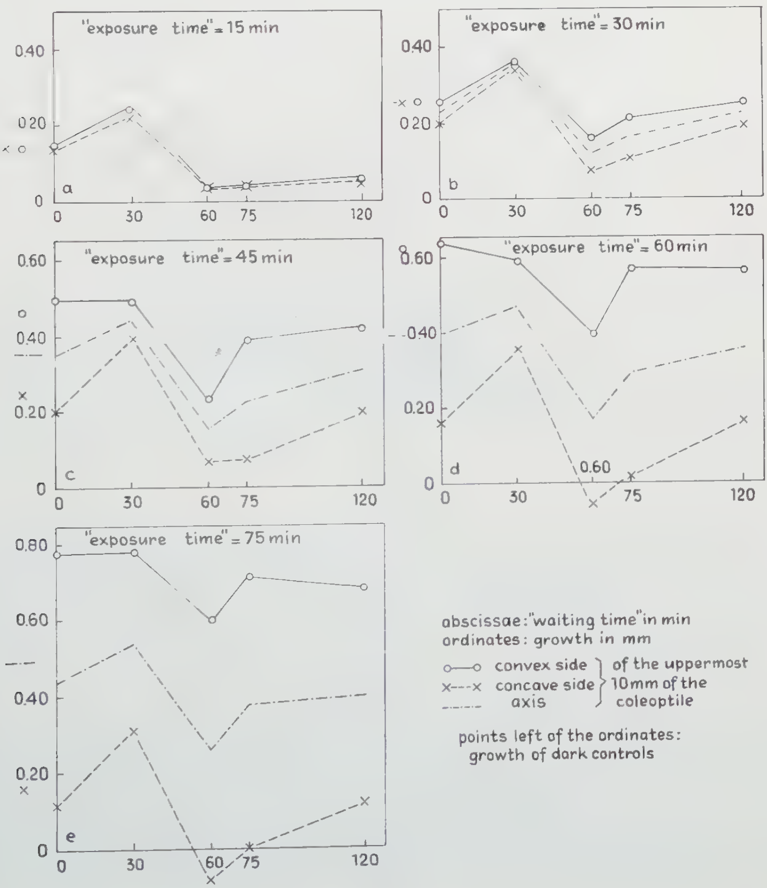

Fig. 27.

The result of this experiment in so far as the first 12 trays are concerned are shown in the graphs of Fig. 27 (growth) and 28 (curvature).

Growth and curvature of the dark controls are given in the points left of the ordinates.

The plants growing vertically showed the behaviour already described in graphs *a* and *e* of Fig. 19.

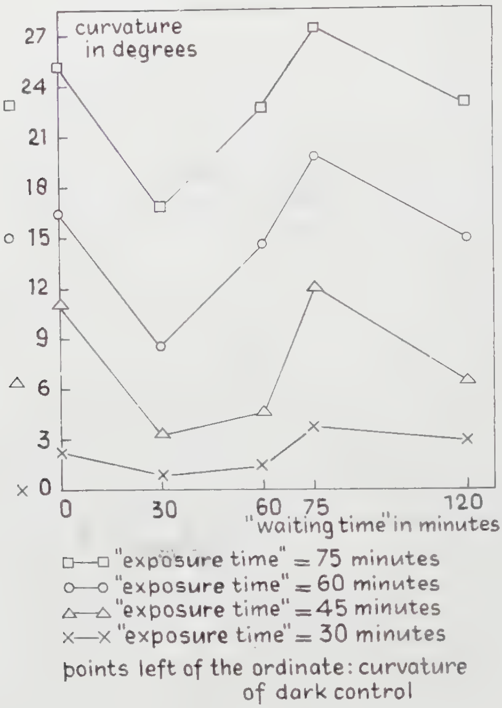

Fig. 28.

In the graphs of Fig. 27, *b*, *c*, *d*, *e* the growth of the longitudinal axis of the coleoptile, being the mean of the growths of the concave and convex sides, is given also.

Secondly an experiment was performed using 36 trays, containing c. 12 plants each.

A first series of 18 trays was irradiated with 500,000 erg cm^{-2} of blue light ($\lambda = 479$ mμ). After the irradiation, the plants were placed in darkness for three hours (in vertical position). After these three hours has passed, 16 of the 18 trays were irradiated with 2700 erg cm^{-2} of blue light and put horizontal in pairs, 0, 15, 30, 45, 60, 75, 90 and 120 minutes resp. after this irradiation. The two remaining trays were put horizontal without a second irradiation. After an "exposure time" of 60 minutes the curvatures were measured in the usual way.

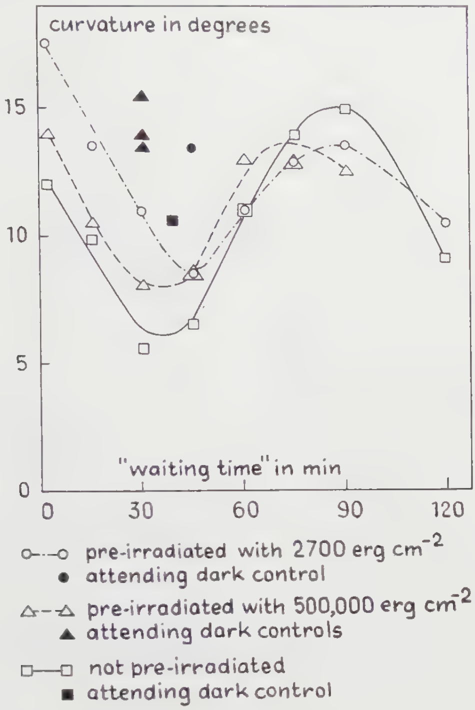

Fig. 29.

A second series of 18 trays was treated in exactly the same way, with the only difference that with the first irradiation a quantity of 2700 erg cm^{-2} of blue light was applied.

As will be clear from the graphs of Fig. 29, generally speaking, it can be said that the plants have "forgotten" their first irradiation after three hours have passed.

CHAPTER XIII

THE INFLUENCE OF BLUE, RED AND FAR RED LIGHT ON THE GROWTH OF THE SEEDLING, EXCLUDING ITS TOP-MOST 10 mm (REFERRED TO AS: "GROWTH OF MESOCOTYL")

In the experiments described in Chapter IX, Fig. 19 and 20, the growth curve of the mesocotyl after irradiation with a number of light quantities of various wave lengths was given.

In the experiments with combined irradiations shown in Fig. 24, the growth curves of the mesocotyls were also measured, but, for reasons of surveyability the data were not presented in Fig. 24, but

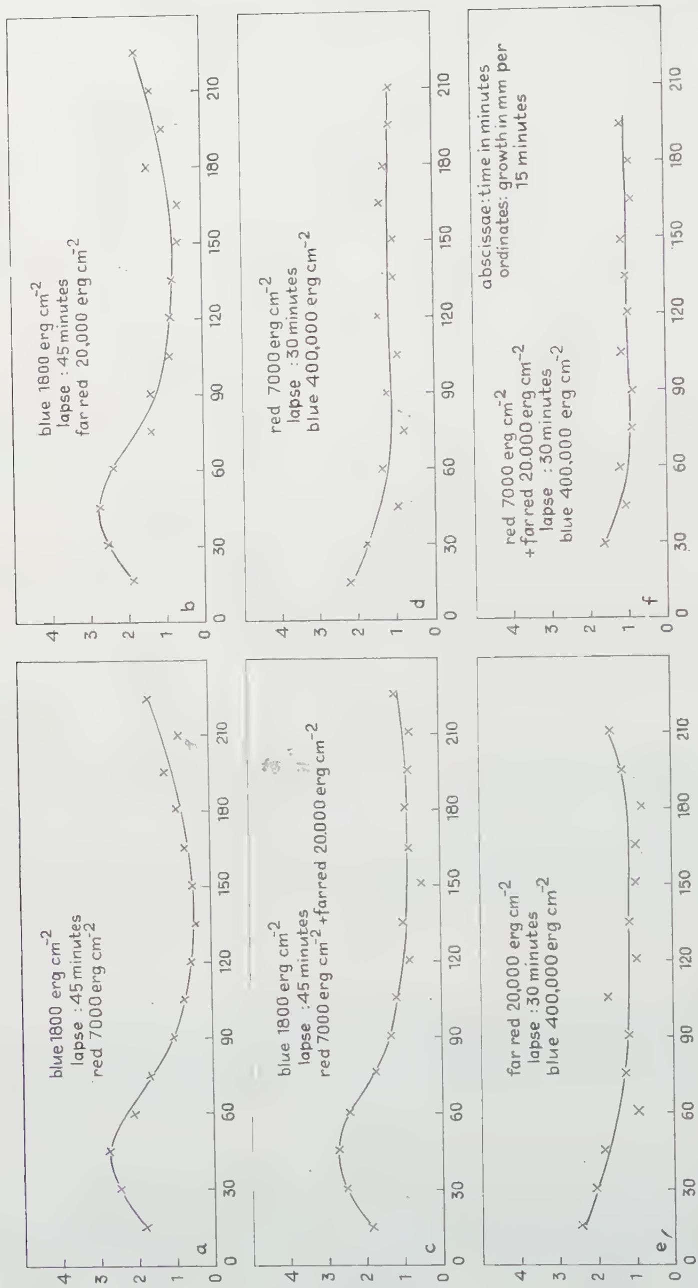
blue 1800 erg cm^{-2}
lapse : 45 minutes
red 7000 erg cm^{-2}
blue 1800 erg cm^{-2}
lapse : 45 minutes
far red 20,000 erg cm^{-2}
blue 1800 erg cm^{-2}
lapse : 45 minutes
red 7000 erg cm^{-2} + farred 20,000 erg cm^{-2}
red 7000 erg cm^{-2}
lapse : 30 minutes
blue 400,000 erg cm^{-2}
far red 20,000 erg cm^{-2}
lapse : 30 minutes
blue 400,000 erg cm^{-2}
red 7000 erg cm^{-2}
+ far red 20.000 erg cm^{-2}
lapse : 30 minutes
blue 400,000 erg cm^{-2}
abscissae: time in minutes
ordinates: growth in mm per 15 minutes
a
b
c
d
e
f

Fig. 30.

will follow in Fig. 30 *a-f*. The dark controls are the same as that of Fig. 19.

From Fig. 19*e* and 30*a*, *b* and *c* it can be seen, that, from the pre-irradiations investigated, only a pre-irradiation with 1800 erg cm^{-2} of blue light induces a growth response in the mesocotyl. In all other cases a decreased growth of the mesocotyl after pre-irradiation is observed.

Apparently the reaction upon irradiation of the mesocotyl is not correlated to that of the coleoptile in a simple manner. The phenomena described may be discussed in comparison with data on the growth of mesocotyls, known from literature.

Mer (1951) examined the possibility whether the growth of the mesocotyl is regulated by the auxin produced in the coleoptile. The results of his experiments exclude this possibility. In 1959 he concluded from experiments on the influence of CO_2 on the growth of the *Avena* seedling, that the growth of the mesocotyl and that of the coleoptile are correlated by the food supply from the seed.

If one assumes, that irradiation influences the geotropic reaction via the indole-3-acetic acid metabolism, our results, revealing no correlation between the reactions of the mesocotyl and the coleoptile, might be in agreement with Mer's findings in 1951. Pre-irradiation with 1800 erg cm^{-2} of blue light may influence the food distribution in the seedling. This would be in agreement with the view that in some cases irradiation influences the transport system of the seedling.

CHAPTER XIV

GENERAL DISCUSSION

Since in phototropism, tropistic and tonic effects are intermingled and phototropism therefore is difficult to explain, the present author had proceeded in such a way that the tonic effects are caused by light only and the tropistic effects are obtained by gravitational stimulation exclusively. In this way he hoped to deepen the insight in phototropic and geotropic phenomena. The results of his experiments, however, are cause of his loosing every mainstay.

Geotropic phenomena are usually met in terms of indole-3-acetic acid or of a co-factor to IAA (Brauner and Hager, 1958). With respect to phototropic phenomena Blaauw-Jansen (1959) discussed the interaction of indole-3-acetic acid and a "red light factor". The existence of the latter she demonstrated in the *Avena* coleoptile.

Now from experiments described in this paper (Fig. 19*f*) it becomes evident that large quantities (400,000 erg cm^{-2}) of blue light increase the growth rate of the coleoptile and that, three hours after irradiation, the coleoptile behaves as if never irradiated, at least with respect to growth (Fig. 19*e*) and geotropic reaction (Fig. 29), in darkness as well as when irradiated once more.

Oppenoorth (1941) performing estimations of the auxin contents

of coleoptiles after irradiation with blue light (26,400 erg cm^{-2}, $\lambda = 436$ mμ) found a gradual increase of the auxin contents. This increase had not yet reached its maximum three hours after the irradiation. Analogous results he found after irradiation with 3000 erg cm^{-2} ($\lambda = 436$ mμ).

According to Oppenoorth irradiation with 330 erg cm^{-2} ($\lambda = 436$ mμ) causes a decrease in the auxin contents, but Fig. 19*d* of this paper shows that the growth of the coleoptile is not influenced after an irradiation with a quantity of blue light of this order of magnitude.

The growth of the coleoptile, therefore, obviously shows no parallel with the auxin contents as estimated by Oppenoorth.

Terpstra (1953) has critisized the method of extraction used by Oppenoorth. It seems clear from her investigations, that Oppenoorth not only estimated the "really free auxin" (present in "negligible" quantities), but also part of the "auxin complex" and part of the auxin that can be formed from "auxin precursors" during the extraction process. Oppenoorth performed the extraction by immersing the coleoptiles in ether of 4° C for at least five hours. Terpstra proposed a mode of extraction by which only "free auxin" and "auxin complex" should be determined, viz. grinding, freezing and subsequently extracting with water of 4° C. According to these procedure Blaauw-Jansen (1962) estimated the auxin contents of coleoptiles 90 minutes after irradiation with 2700 erg cm^{-2}, 120 minutes after irradiation with 2700 erg cm^{-2} and 60 minutes after irradiation with 400,000 erg cm^{-2}. All irradiations were performed with blue light ($\lambda = 479$ mμ). In all cases she found a decrease of the auxin contents to $\frac{1}{2}$ or $\frac{1}{4}$ approximately of the contents of the dark controls.

The results of this method of extraction do not show any parallel between the quantity of auxin extracted and the growth response of the coleoptiles reported in this paper. Yet it is Terpstra's opinion that the "auxin complex" is responsible for growth.

If one has no intention to drop the idea that auxin has something to do with growth and geotropic curving, one is forced to assume that the extraction methods described do not lead to the extraction of the auxin-fraction responsible for the phenomena mentioned in this paper. It does not seem to lead any further to discuss the available data on auxin contents in this connection.

The lateral transport of auxin is another topic in literature concerning geotropism and phototropism. At first the present author was inclined to the view, that small as well as large quantities of blue light in some cases hamper the lateral transport. E.g. after an irradiation with 2700 erg cm^{-2} of blue light and applying a "waiting time" of 30 minutes a decreased geotropic reaction, combined with an increased growth of the concave side of the coleoptile, is observable. This would lead to the assumption that under these circumstances the growth of the convex side should be less than normal, presuming that the growth measured along the longitudinal axis is not influenced. From a number of experiments, however, most clearly from the experiment of Fig. 27, it can be seen that under the circumstances

described, the growth measured along the longitudinal axis of the horizontal coleoptile is considerably enhanced and so is the growth of the convex side. This leads to a closer contemplation of the data from which it is possible to compare the growth of convex side, concave side and longitudinal axis of the horizontally growing coleoptile, with the growth of the vertical coleoptile. These data are compiled in the graphs of Fig. 12, 13, 19, 24, 25 and 27, which are all in agreement with each other.

Fig. 12, 13 and 27 show the growth of the concave side, the convex side and the longitudinal axis of the coleoptile.

There seems to be no correlation between the growth of the longitudinal axis and the curvature. The facts are that after various pre-irradiations and "waiting times", low curvatures are attended with an increased growth of the concave side, high curvatures with an increased growth of the convex side and medium curvatures with an increased growth of both the concave and convex sides, or with growth rates that do not differ from that of the dark controls. In one case (Fig. 13*e*), the increased curvature is attended with a very strongly increased growth of the convex side and an increased growth of the concave side of the coleoptile.

From Fig. 12*b*, *c* and *d*, but most clearly from Fig. 27 it can be seen that after pre-irradiation with 2700 erg cm^{-2} of blue light ($\lambda = 479$ $m\mu$) a diminished curvature is attended with an increased growth of the longitudinal axis, an increased curvature with a decreased growth of the axis. The most pronounced increase of the curvature ("waiting time" = 75 min), however, is not attended with the most pronounced decrease in growth of the axis; the latter occurring at a "waiting time" of 60 minutes.

In most cases shown in Fig. 12 and 13, perhaps with the exception of those given in Figs. 12*d* and 13*e*, the growth of the longitudinal axis of the horizontal coleoptiles is less than that of the comparable vertical ones. A similar effect of gravity on the tonus of coleoptiles is described by ANKER (1960) who demonstrated that in some instances a coleoptile that is turned every ten minutes from one side to the other, so as to be continually influenced by unilateral gravity, without being allowed to curve, shows a decreased growth compared to a coleoptile growing in the upright position. This geo-growth reaction occurred with non-decapitated coleoptiles as well as with decapitated ones that were immersed in solutions containing 0.05 mg/l of indole-3-acetic acid, but could not be demonstrated with decapitated coleoptiles immersed in solutions containing 0 or 0.2 mg/l. It should be mentioned that Anker's experiments were performed while using orange light (Schott OG2) as a dark room illumination.

As to the explanation of our finding of the two-peaked curves of Figs. 14*c*, *d*, *f*, *g*, *i*, and *k* (schematically reproduced also in Fig. 16), we attempted to correlate them with the phenomena described by Soekarjo and by Blaauw-Jansen. SOEKARJO (1961) demonstrated that at low IAA concentrations a maximum appears in the curvature v. IAA concentration curve of petioles of *Coleus sp.* BLAAUW-JANSEN

(1959) observed the following phenomena with *Avena* coleoptil
At low IAA concentrations, after an irradiation with red light, s
observed an increased growth of coleoptile sections that can
annihilated by a subsequent irradiation with far red light. Also,
low IAA concentrations, she found a maximum in the curvature
IAA concentration curve, when, after irradiation with red light
curvature of coleoptiles was measured in the standard Avena t
(Fig. 23). From coleoptiles that were irradiated with red light, Blaau
Jansen was able to isolate a substance (the "red light factor"), exert
the same influence as red irradiation does. This substance proba
also occurs in non-irradiated coleoptiles, but in a smaller quantity th
in irradiated ones. In the light of these findings she was able to prop
an attractive elucidation of the phenomena of first positive, negat
and second positive phototropic curvature, as a gradual decre
of the IAA contents of the coleoptile is attended with a fall, a
and another fall of the growth rate (Fig. 31).

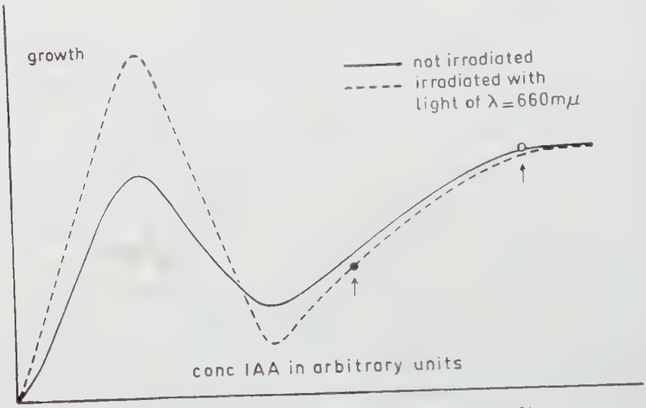

Fig. 31. (from BLAAUW-JANSEN, 1959)

From her considerations it is clear, however, that in her experim
90 minutes after irradiation, the tonic condition of the plant sh
essentially differ from that of non-irradiated plants.

In the experiment, described in Fig. 29 of this paper, we demon
ted that the tonic state of the plant, three hours after irradiatio
the same as that of non-irradiated plants. Though it seemed po
to correlate Blaauw-Jansen's considerations mentioned above, O
oorth's findings concerning the decreasing auxin contents
irradiation and our finding of the two-peaked curves of Fig. 14
possibility vanished in the light of the experiment described in F
of this paper.

From Figs. 12*e*, *f*, *h*, *i*, and *j* it is obvious, that the increase
curvation produced after irradiation with red light ("waiting tim
30 min) is attended with an enhanced growth of the convex side
coleoptile and with a reduced growth of the concave side. Th

that the influence of pre-irradiation with red light on the geotropic reaction is not observable after a "waiting time" of 60 minutes cannot be ascribed to a restoration of the original situation, as after a "waiting time" of 60 minutes the growth of the concave as well as of the convex side of the coleoptile and concurrently the growth of the longitudinal axis is enhanced, and the same is true for the growth of the vertical coleoptile in those circumstances. Moreover, BLAAUW-JANSEN (1959) demonstrated that in experiments concerning phototropism, the influence of pre-irradiation with red light was observable up to six hours after its administration.

It is remarkable too, that the stimulation of the geotropic reaction by red light ("waiting time" = 30 min) (Fig. 12*f* and *i*) shows the same pattern of growth influences as the stimulation by 400,000 erg cm^{-2} of blue light does ("waiting time" = 10 min) (Fig. 13*e*). Red light, however, in contradistinction to blue light, shows no phototropic activity.

The influence of small quantities of blue light (2700 erg cm^{-2}) seems to be of another nature. From Fig. 6 it can be seen that with low quantities of blue light, the influences of red and blue light are additive; with high quantities of blue light, the influence of red light is absent or, at the least, less pronounced. This leads to the assumption, that much blue light is comparable to little blue light + red light. To check the correctness of this assumption, it would be necessary to try whether the curve of Fig. 14*d* can be transformed in that of Fig. 14*i* by pre-irradiation with 1800 erg cm^{-2} of blue light + 7000 erg cm^{-2} of red light, applying the "waiting times" mentioned in these figures. This, however, does not seem likely.

In all the cases investigated, far red light (λ = 735 mμ) exerts the same influences as red light (λ = 660 mμ) does, when administered in combination with various quantities of blue light as well as in combination with red light, or when administered by itself. No red-far red antagonism could be observed.

From our experiments and our attempts to discuss them it becomes clear, that the interpretation of the phenomena described is severely complicated by the fact that unilateral gravity itself has not only a tropistic effect on the coleoptile, but a tonic effect as well, and therefore the present author has not succeeded in his intention to disentangle tonic and tropistic influences.

The only conclusion is, that it seems impossible to interpret the tonic and tropistic phenomena in the terms of the conventional auxin theory.

SUMMARY

Seeds of *Avena sativa* were husked, wetted, germinated for 19 hours on moist filter paper, during which period they were irradiated with orange light (Schott OG2) and subsequently grown in total darkness in moist vermiculite for c. 65 hours. Then they were irradiated with violet, blue, red or far red light (interference filters of λ=415, 479, 660 and 735 mμ resp.) and at once, or some time after the irradiation the growth and the geotropic curving reaction (further referred to as: "geotropic reaction") of the coleoptiles and in some cases the growth of the whole plants, were measured.

For necessary manipulations and for shadowgraphing, very dim green light (interference filter of $\lambda = 560$ mμ) was used.

Irradiations were given from directly above, unless otherwise stated.

As light intensities of c. 1000 erg cm^{-2} sec^{-1} were at our disposal, light quantities up to 500,000 erg cm^{-2} could be administered within a period of ten minutes.

Each of the pre-irradiations mentioned above exerts an influence on the growth of the coleoptile and on the geotropic reaction. This influence is only marked during a relatively short period, occurring a shorter or longer time after the irradiation. In practically all cases, the "exposure time", i.e. the time lapse between the moment of putting the plants horizontal and the moment of registration of the reaction, was one hour. The time lapse between the beginning of the irradiation and the moment of placing the plants horizontal was termed "waiting time".

Red light only

Quantities from 4 up to c. 7000 erg cm^{-2} of red light exert an increasing influence on the geotropic reaction. Larger quantities (up to 800,000 erg cm^{-2}) show the same effect as 7000 erg cm^{-2} does.

As compared to dark controls, the coleoptiles show a number of effects of pre-irradiation with red light, that are compiled in the third part of table 26. The effects mentioned in table 26, are the effects occurring during a period of 60 minutes immediately after the "waiting time".

TABLE 26

"Waiting time"	curvature	growth of the vertical coleoptile	growth of the horizontal coleoptile: convex side	growth of the horizontal coleoptile: concave side	growth of the horizontal coleoptile: longitudinal axis
			Little blue light (1000–10,000 erg cm^{-2})		
0 min.	0	0	0	0	0
30 min.	– –	0	0	+ +	+
60 min.	0	—	—?	—?	—?
75 min.	+	0	—	– –	—
120 min. or more	0	0	0	0	0
			Much blue light (100,000–500,000 erg cm^{-2})		
0 min.[3])	+ +	+	+ +	+	+ +
30 min.	0	+	+ +	+ +	+ +
60 min.	0	0	0	0	0
75 min.	+	0			
120 min. or more	0	0			
			Red or far red light (7000 or 20,000 erg cm^{-2} resp.)		
0 min.	0	—[1])	0	0	0
30 min.	+ +	+	+	0	+
60 min.	0	+	+	+	+
75 min.		+			
120 min. or more		+[2])			

1) A decrease followed by a restoration.
2) A gradually diminishing increase.
3) "Waiting time" > 0 min; the time, necessary for illumination, amounting to ten minutes.

0 = no effect
\+ = increase
\+ + = strong increase
— = decrease
– – = strong decrease
? = no clear-cut effect

Far red light only

Essentially the same pattern of influences as that of red light is obtained with far red light, except for the fact that the quantities needed are about three times higher.

Blue light only

The influence of relatively small quantities of blue light (1000—10,000 erg cm^{-2}) seems essentially different from that of large quantities (100,000—500,000 erg cm^{-2}).

The effects of small and large quantities of blue light as compared with dark controls, measured after various "waiting times" are compiled in the first and second part of table 26. Here too, the effects mentioned are the effects that occur during the period of 60 minutes immediately following the "waiting time".

Violet light only

In the few cases that were investigated, small and large quantities of violet light showed the same pattern of influences as was observed with blue light, only somewhat larger quantities of violet light were necessary to obtain effects comparable with that of the pre-irradiation with blue light.

Combinations of irradiations

Generally speaking it can be said that in the investigated cases, the effects of irradiation with blue + red light are additive, except for very large quantities of blue light. Each of the irradiations exerts its own influence, with respect to light quantity as well as to "waiting time". The same seems to be true for blue + far red light.

In several ways it has been attempted to demonstrate red - far red antagonism, which has not been found.

Phototropism and geotropism

Pre-irradiation with a range of quantities of blue light exerts the same influence on the geotropic reaction as is observed after pre-irradiation with the same range, administered from two sides.

When this range is administered unilaterally, first positive, negative and second positive phototropic curvatures are induced.

The curvature v. light quantity curve of the geotropic reaction shows no correlation with that of the phototropic reaction.

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THE EFFECT OF AUXIN AND SUCROSE ON GROWTH AND FORM OF PEA STEM SECTIONS

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INTRODUCTION

The results, reported in literature, of experiments on the effect of indoleacetic acid, in the presence of sucrose, on the growth of excised sections of pea epicotyls are rather inconsistent. GALSTON and HAND (1949) and GALSTON and BAKER (1951) report that sucrose enhances the growth of etiolated epicotyl sections in the presence of indoleacetic acid. CHRISTIANSEN and THIMANN (1950) found no effect of sucrose on growth of etiolated sections. With pea stem sections in light, BRIAN and HEMMING (1958) obtained increased growth by the addition of 2 % sucrose to near-optimal concentrations of indoleacetic acid. In the experiments of GALSTON and BAKER (1951) sucrose retarded the growth of sections in light.

These inconsistencies have been ascribed to different causes. AUDUS (1952) has pointed out that, under different conditions, the time course of growth may be different. If only total growth is determined the result obtained may depend upon the duration of the experiment. DE ROPP and MARKLEY (1950) found that sections from the apical and from the basal end of sunflower hypocotyls reacted in a different way to indoleacetic acid; PURVES and HILLMAN (1958) reported that sections of pea epicotyls of different length reacted differently.

In several investigations it has been found that, in the presence of sucrose, the optimal concentrations of indoleacetic acid for growth in length and for increase in fresh weight are different (e.g. GALSTON, BAKER and KING, 1953; DE ROPP and MARKLEY, 1953). This may give rise to confusion, for increase in weight has been used as a measure for growth in length (AUDUS, 1952) as well as for lateral growth (PURVES and GALSTON, 1960).

It seems difficult to understand why the optimal concentrations of IAA for longitudinal and for lateral growth are different. PURVES and GALSTON (1960) have suggested that these types of growth were not independently affected by auxin, but that high lateral growth will inhibit growth in length. In work in our laboratory, with the dwarf pea variety "Rondo", a rather uncommon curve of the effect of IAA concentration in the presence of sugar on longitudinal growth was obtained. We believe that this curve illustrates the dependency of longitudinal growth on lateral extension in a better way than most curves, that have been published.

Material and Methods

Peas of the dwarf variety Rondo were soaked in tap water for 3–4 hours. The seeds were sown in pans containing vermiculite. The pans were placed in a dark room at 28° C. The light that was necessary when handling the plants came from a 80 W incandescent bulb screened by a green glass. After 6–7 days the third internode grew out.

For the experiments plants were chosen the third internode of which had a length of 2–4 cm. By means of a cutter consisting of two parallel razor blades a cylinder of 5.1 mm. was cut from the third internode at a distance of 1 mm. from the tip. The cylinders were floated on the surface of the solutions in 50 ml. glass beakers. The solutions always contained .02 M. phosphate buffer of a pH of 5.9–6.0. The solutions were stirred by a gentle air stream. After 24 hours the sections were placed in holes which had been drilled in a perspex block. The block was placed under water in a cuvette in front of an incandescent lamp. By means of a lens system the shadow of the sections was projected on the wall. The enlargement was exactly ten times. The length and the largest diameter of the sections were measured with a ruler. Unless stated otherwise the figures in the tables are the average of the measurements of 10 sections. For microscopical inspection the sections were fixed in Rawlin's solution: alcohol 50 % 100 ml., formalin 40 % 6.5 ml., glacial acetic acid 2.5 ml.

Results

1. *Effect of auxin and 2 % sucrose*

The effect on growth of different concentrations of indoleacetic acid in buffer solution and of the same concentrations in the presence of 2 % socrose is shown in Fig. 1. The data from which the curves have been drawn are given in table 1 and in tables 2–4. The numbers in figure 1 give the water absorption of the sections. This was determined by weighing of the sections before and after the experiment. In the experiments with 2 % sucrose water absorption has been determined in 11 experiments, viz. in expts nrs 34–45.

Fig. 1 shows that water absorption is not always a reliable measure for lateral growth. For instance, the diameter of sections grown in 2 % sucrose without auxin and of sections grown in 2 % sucrose in the presence of 10^2 μg./ml. of indoleacetic acid is the same (2.3 mm.) while the water absorption is different (58 % and 114 %).

Lateral growth is only slightly affected by indoleacetic acid when sucrose is absent. Under these conditions growth in length is enhanced by concentrations of auxin between $10^{-2}\mu$g./ml. and 10 μg./ml. A concentration of 10^2 μg./ml. is supra-optimal. A similar flat curve for the effect of indoleacetic acid on longitudinal growth of Avena sections has been published by Schneider (1938).

Lateral growth is enhanced by indoleacetic acid in the presence of 2 % sucrose. The effect is maximal at concentrations of 1 μg./ml. and 10 μg./ml. At these concentrations the sections become barrel-shaped owing to the fact that lateral growth is higher in the middle than at

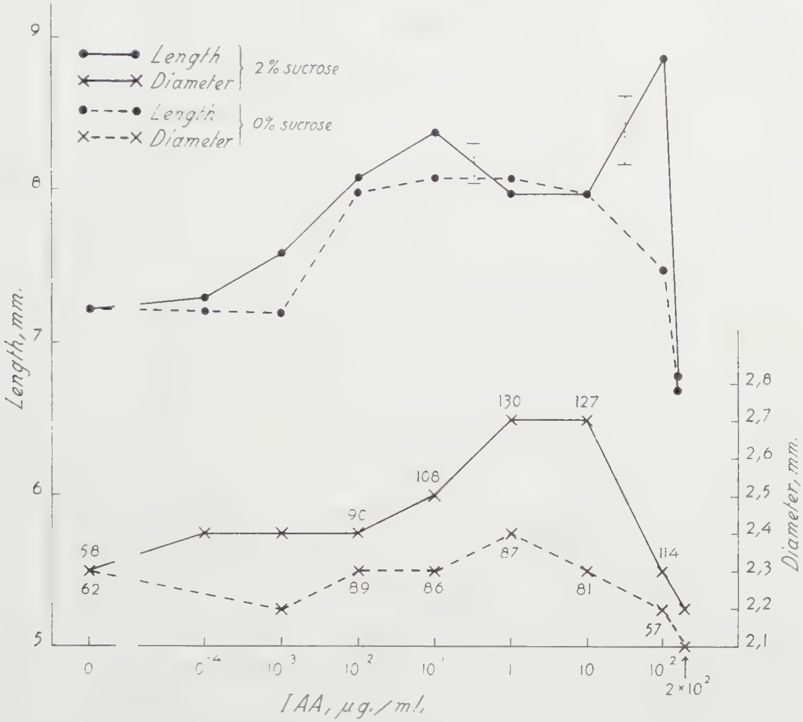

Fig. 1. Effect of indoleacetic acid on growth in the absence of sucrose and in the presence of 2 % sucrose. The two dotted vertical lines indicate the least significant difference between longitudinal growth in the presence of 2 % sucrose at 10^{-1} μg/ml and 1 μg/ml and at 10 μg/ml and 10^2 μg/ml respectively. (P = 0,01). The numbers indicate the increase in fresh weight in percents of the original weight.

both ends. The same phenomenon has been reported by Purves and Galston (1960).

The curve which represents the effect of auxin concentration on longitudinal growth shows two peaks separated by a depression. This depression is situated at concentrations of indoleacetic acid that are optimal for lateral growth. The difference between the maximum at $10^{-1}\mu$g./ml. and the decreased growth at 1 μg./ml. of I.A.A. is 0.35 ± 0.1 mm. The difference between the growth at 10 μg./ml. and the second growth maximum at 10^2 μg./ml. is 0.83 ± 0.18 mm. These differences are highly significant.

2. *Effect of auxin and* 1 % *sucrose*

In the presence of 1 % sucrose the effect of indoleacetic acid was not the same in different experiments. Probably the state of the plants, e.g. the sugar content of the cells, affected the results.

Fig. 2 shows the result of one experiment. The diameter of the sections was maximal at 1 μg./ml. of IAA, as in the experiments with

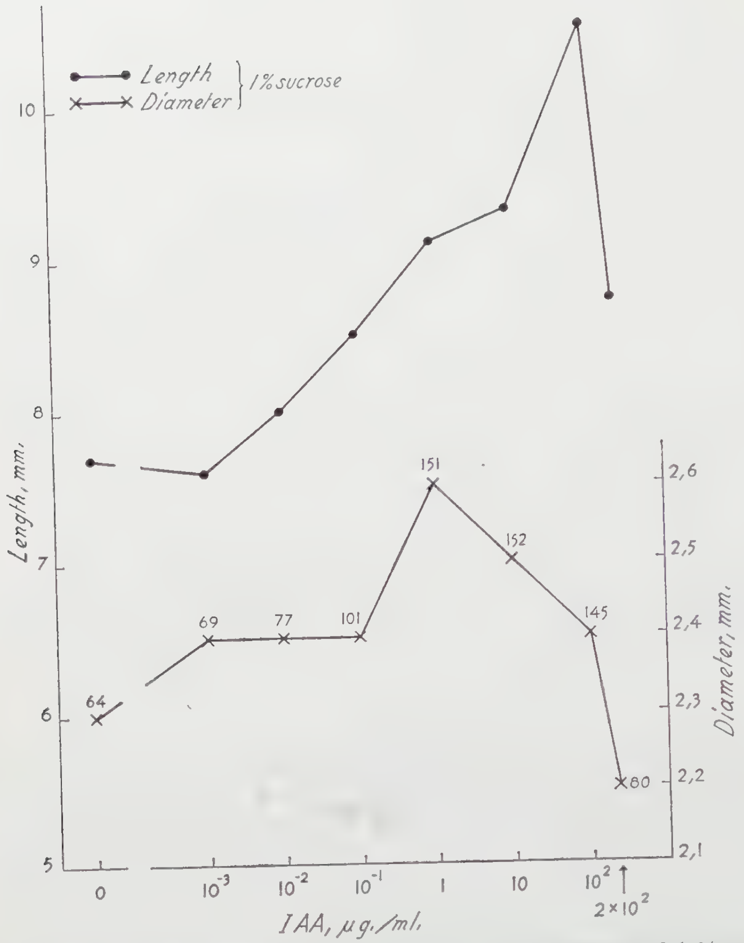

Fig. 2. Effect of indoleacetic acid on growth in the presence of 1 % sucrose. The numbers indicate the increase in fresh weight in percents of the original weight.

2 % sucrose, but the maximum is .1 mm lower. From 1 μg./ml. towards higher concentrations the curve representing the diameter falls off. In the curve of longitudinal growth a depression is practically absent. The curve shows an optimum at 10^2 μg./ml.

In a second experiment the effect of 1 % and 2 % of sucrose was compared at concentrations of indoleacetic acid that cause the depression in the curve of longitudinal growth. Fig. 3 shows the results. In this experiment growth in length was decreased at 1 μg./ml. and at 10 μg./ml. of IAA in the presence of both, 1 % and 2 % sucrose. The diameter of the sections was maximal at these concentrations. In 2 % sucrose the depression in the curve of longitudinal growth is deeper and the maximum of the curve representing the diameter of the sections is higher than in 1 % sucrose.

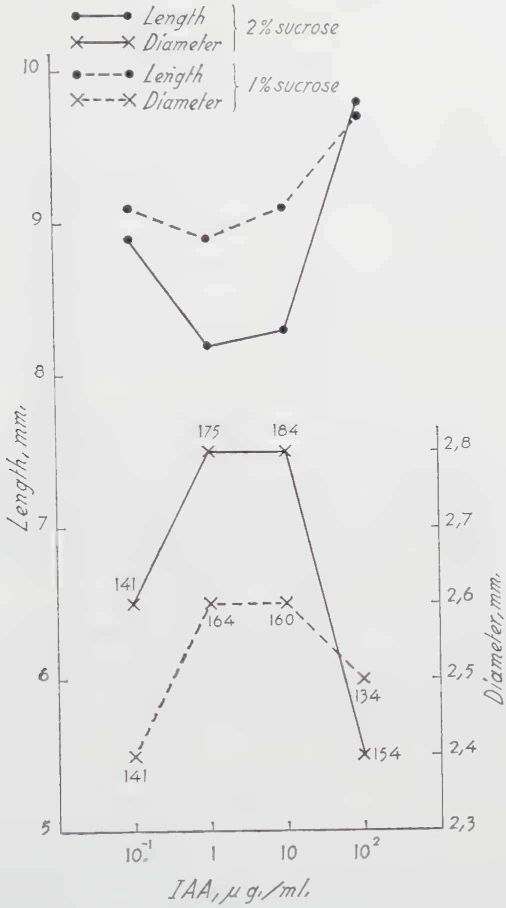

Fig. 3. Effect on growth of 1 % and of 2 % of sucrose in the presence of 4 con centrations of indoleacetic acid. The numbers indicate the increase in fresh weight in percents of the original weight.

Discussion

The present results differ in mainly one respect from those obtained by previous authors, viz. the effect of auxin concentration on growth in length in the presence of 2 % sucrose. Usually, the graph representing this relationship shows one optimum. In the present experiments a curve with two maxima, separated by a depression, was obtained. The optimum for lateral growth in the presence of 2 % sucrose lies at the same concentrations of auxin as the depression in the curve representing longitudinal growth. The following explanation is suggested for this coincidence.

It is fairly generally agreed upon that auxin induces a weakening of the cell wall. This causes the cell to absorb water osmotically and the osmotic pressure of the cell to decrease till it attains a new equilibrium with the tension of the cell wall. If the cell wall expands in

a longitudinal direction only, the volume of water absorbed is used for longitudinal growth. But if the cell wall expands in both, longitudinal and lateral direction, a considerable portion of the water taken up is used for lateral growth and, hence, the growth in length is decreased. A similar explanation was suggested by Purves and Galston (1960) for the decreased longitudinal growth at high auxin concentrations. Since in their experiments, at the highest auxin concentration applied, lateral growth remained high, growth in length remained low.

A different explanation for the shift by the addition of sucrose of the optimum for longitudinal growth is possible, viz. that sucrose interferes in a direct way with the reactions leading to longitudinal growth. Purves and Galston (1960) mention this possibility. However, it is practically ruled out by the present results. For if in 2 % sucrose the optimal concentration of IAA for lateral growth is too high for optimal growth in length, it is difficult to understand why at still higher concentrations longitudinal growth increases again. It must be concluded, therefore, that the shifting of the optimum for longitudinal growth found by Purves and Galston and the depression in that curve found in the present experiments are the result of increased lateral growth.

It may be asked why longitudinal growth takes place at a considerable rate in concentrations of auxin that are too low or too high to cause much lateral growth. Castle (1937) and Diehl, Gorter, Van Iterson and Kleinhoonte (1939) demonstrated that if, in a cylindrical cell, the strength of the cell wall is equal in all directions lateral extension will predominate over longitudinal. The fact that the cells of the young stem grow mainly longitudinally proves that the resistance of the cell wall in lateral direction is higher than in a direction parallel to the longest axis. The structure of the cell wall responsible for this property has been described by Frey-Wyssling (1959).

We suggest that a relatively low rate of the auxin-induced reactions will be suffcient to decrease the low resistance of the cell wall to longitudinal extension, and that a high auxin activity is required to decrease the resistance of the wall to lateral extension. This hypothesis explains why lateral growth takes place at a narrow range of near-optimal concentrations only. At these concentrations the lateral growth will inhibit longitudinal extension by the mechanism described above. At concentrations of indoleacetic acid that are farther away from the optimum auxin activity in the cell will be too low to induce weakening of the cell wall in lateral direction but it may be still high enough for longitudinal extension. For that reason growth in length will be highest at concentrations that are just too high or just too low for lateral growth to take place.

The hypothesis that the rate of lateral growth is high only when auxin activity is high implies that not only concentration, but also any other agent that increases auxin activity may induce increased lateral growth. This might be a simpler explanation for the effect of benzimidazole on lateral extension than the hypothesis of a selective

effect of this substance on certain areas of the cell wall (GALSTON, BAKER and KING, 1953).

SUMMARY

1. In the presence of 2 % sucrose the curve representing the effect of the concentration of indoleacetic acid on the growth in length of etiolated pea stem sections of the variety "Rondo" shows two maxima separated by a depression.

2. The depression in the curve of longitudinal growth coincides with the optimum for lateral growth.

3. It is concluded that high lateral growth inhibits growth in length.

4. It is suggested that, when auxin activity in the cell is low, the cell wall is weakened mainly in longitudinal direction and that at higher auxin activities the cell wall is weakened in all directions.

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TABLE 1

Length, diameter and water absorption of sections in phosphate buffer ar indoleacetic acid after 24 hours. Initial length: 5.1 mm

Conc. of IAA in μg/ml	0	10^{-3}	10^{-2}	10^{-1}	1	10	10^2	2×1
Average length in mm	7,2	7,2	8,0	8,1	8,1	8,0	7,5	6,7
Average diameter in mm	2,3	2,2	2,3	2,3	2,4	2,3	2,2	2,1
Waterabsorption in % of original weight .	62		89	86	87	81	57	

TABLE 2

Length of sections in mm in the presence of 2 % sucrose, indoleacetic acid ar phosphate buffer after 24 hours. Initial length: 5.1 mm. The figures represent t average length of the number of sections indicated in the second column

Conc. of IAA in g/ml		0	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10	10^2	2×1
Expt. number	Number of sections									
8	10	7,2	7,0	7,5	7,8	8,2	8,0	7,8	8,5	6,
10	10	6,7		7,8	8,4	7,4	8,1	8,2	6,6	6,
11	10	7,3	8,7	9,4	9,2	9,6	8,5	8,6	10,2	7,
12	10	6,8	7,0	7,6	7,1	8,1	8,4	8,4	7,6	6,
14	10	7,3	7,0	7,3	7,9	8,3	8,1	8,0	8,6	6,
15	9	6,7	6,7	6,8	7 3	7,4	7,9	8,5	8,7	6,
16	10	7,6	7,4	8,2	8,7	9,5	8,2	8,0	9,2	7,
17	7	7,0		7,1	8,8	9,0	7,9	8,3	8,8	6,
18	10					8,4	8,0	8,4	9,3	
18	10					8,5	8,3	8,4	9,3	
19	7				7,5	8,3	7,9	8,1	8,7	6,
20	10	7,8	7,3	7,7	7,9	7,8	7,8	7,7	8,6	6,
22	10	6,7		6,5	8,0	7,9	7,4	7,9	8,1	6
25	9	7,6		7,9	8,3	8,9	8,8	8,2	9,9	7
32	10	8,3		7,8	8,5	8,8	8,0	8,1	9,7	8
33	10					8,1	8,2	7,1	9,3	
34	9					9,3	8,3	8,1	9,2	
35	7					7,9	7,4	7,6	8,4	
36	5					7,9	7,7	7,6	9,0	
37	10					7,9	7,6	7,9	9,3	
38	10					7,8	7,8	7,8	8,5	
42	10					8,9	8,2	8,3	9,8	
44	7	7,1			8,1	8,4	8,1			
45	8	7,4			8,5	9,0	8,3			
Total:		101,5	51,1	91,6	122,0	201,3	192,9	177,0	195,3	88
Average of all the experiments		7,2	7,3	7,6	8,1	8,4	8,0	8,0	8,9	6

TABLE 3

Largest diameter of the sections of table 1 in the presence of 2 % sucrose, indoleacetic acid and phosphate buffer after 24 hours. The initial diameter has not been determined

Conc. of IAA in gr/ml		0	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10	10^{2}	2×10^{2}
Expt. number	Number of sections									
10	10	2,2		2,3	2,5	2,6	2,6	2,6	2,2	2,3
11	10	2,4	2,6	2,7	2,5	2,7	2,8	2,9	2,4	2,2
12	10	2,5	2,4	2,3	2,4	2,5	2,6	2,6	2,3	2,2
14	10	2,5	2,5	2,4	2,5	2,5	2,8	2,6	2,4	2,2
15	9	2,3	2,4	2,4	2,4	2,5	2,7	2,7	2,2	2,1
16	10	2,3	2,5	2,5	2,5	2,5	2,8	2,8	2,4	2,3
17	7	2,2		2,2	2,2	2,4	2,6	2,8	2,4	2,2
18	10					2,6	2,8	2,7	2,5	
18	10					2,3	2,4	2,6	2,1	
19	7				2,4	2,6	2,9	2,9	2,4	2,3
20	10	2,2	2,2	2,2	2,1	2,4	2,4	2,4	2,2	2,0
22	10	2,2		2,2	2,1	2,2	2,4	2,6	2,1	2,0
25	9	2,6		2,6	2,6	2,8	2,8	3,0	2,6	2,3
32	10	2,3		2,3	2,4	2,6	2,7	3,0	2,4	2,3
33	10					2,3	2,5	2,3	2,2	
34	9					2,4	2,5	2,3	2,2	
35	7					2,4	2,6	2,7	2,4	
36	5					2,5	2,8	2,5	2,4	
37	10					2,7	2,9	2,8	2,5	
38	10					2,6	2,8	2,8	2,5	
42	10					2,6	2,8	2,8	2,4	
44	7	2,4			2,6	2,8	3,0			
45	8	2,3			2,5	2,5	2,8			
Total:		30,4	14,6	26,1	33,7	58,0	62,0	56,4	49,2	26,4
Average of all the experiments		2,3	2,4	2,4	2,4	2,5	2,7	2,7	2,3	2,2

TABLE 4

Length and largest diameter of the sections of tables 2 and 3 in the presence of 2 % sucrose, indoleacetic acid and phosphate buffer after 24 hours

Conc. of IAA in g/ml	0	10^{-4}	10^{-3}	10^{-2}	10^{-1}	1	10	10^{2}	2×10^{2}
Average length in mm (from table 1)	7,2	7,3	7,6	8,1	8,4	8,0	8,0	8,9	6,8
Average diam. in mm (from table 2)	2,3	2,4	2,4	2,4	2,5	2,7	2,7	2,3	2,2
Waterabsorption % of original weight	58			90	108	130	127	114	

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THE LOGANIACEAE OF AFRICA[1]
III. SPIGELIA L.

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(*received Aug. 4th, 1961*)

Relationship with other genera

Spigelia L. is the type genus of the *Spigelieae* which are usually herbaceous plants with valvate aestivation, a capsule, and unwinged seeds. It differs mainly from its relatives by a cupula-like base of the capsule which remains after the valves have been shed, and a usually unbranched cincinnous inflorescence.

Geographical distribution

This genus occurs mainly in the tropical parts of America and extends to the north to New Jersey in the United States with one species, *S. marilandica* (L.) L. The majority of species occur in Brazil, and the most widespread, *S. anthelmia* L. occurs in nearly the whole area of the genus, in Florida, Mexico, Central and northern South America, and the West Indies. It is naturalized in Africa and in Indonesia, in Java and the adjacent islands.

Spigelia L., Sp. Pl. 149. 1753; Progel in Martius, Fl. Bras. 6(1): 253. 1868; L. B. Smith, Wrightia 2: 90. 1960.

Annual or perennial herbs or undershrubs, glabrous or hairy with simple or stellate hairs. *Stems* usually terete. *Leaves* simple, opposite, often in whorls or pseudo-whorls at the apex of the stems, with connate sheaths and sometimes interpetiolar stipules. *Inflorescence* usually cincinnous, terminal or nearly so. Flowers opposite the bracts or nearly so. Sepals free or connate at the base, linear-lanceolate to ovate, equal or unequal. Corolla actinomorphic, infundibuliform, frail; lobes 5, erect, valvate in the bud, more or less triangular, acute, shorter than the tube. Stamens 5, included or exserted, equal, alternating with the corolla lobes; filaments free from each other, inserted on the corolla tube; anthers lanceolate or ovate; cells 2, parallel, discrete, dehiscent throughout by a longitudinal split. Ovary superior, 2-celled, with an axial placenta with several ovules in each cell; style 1, of which upper half deciduous and lower half persisting. Fruit a capsule, 2-lobed, loculicidal and septicidal, 4-valved; valves deciduous; a capular base remaining in the persistent calyx. Seeds polyhedrous, more or less obliquely ellipsoid or ovoid, tuberculate, verrucose, or

[1]) Continued from Act. Bot. Neerl. 10: 1-53. 1961 and Meded. Landbouwhogesch. Wageningen 61(4): 1–31. 1961.

reticulate. Embryo straight, surrounded by much endosperm. Colleters in the axils of the leaves, bracts, and sepals (in *S. anthelmia* only?).

Type species: *S. anthelmia* L.

Distribution: About 50 species in tropical and extra-tropical America.

S. anthelmia L., Sp. Pl. 149. 1753; Aublet, Hist. Pl. Guian. Fr. 1: 126. 1775; Poiret in Lamarck, Enc. 7: 344. 1806; Sims, Bot. Mag. 50: t. 2359. 1822; De Candolle, Prod. 9: 7. 1845; E. Bureau, Thèse Logan. 125. 1856; Bentham, Journ. Linn. Soc. 1: 62. 1857; Progel in Martius, Fl. Bras. 6(1): 262. 1868; Solereder in Engl. Prantl, Nat. Pflanzenf. 4(2): 35, f. 17. A. 1892; Backer, Trop. Natuur Ned. Ind. 1: 65. 1912; Heyne, Nutt. Pl. Ned. Ind. 4: 49. 1917; 2nd. ed. 2: 1267. 1927; Hutchinson & Dalziel, Fl. W. Trop. Afr. 2: 25. 1931; v. Raalte in Pulle, Fl. Surinam 4(1): 104. 1932; Fr. Arnoldo, Zakflora Curaçao 98, 119, f. 45. 1954; Saunders, Handb. W. Afr. Flow. 56. f. 183. 1958; L. B. Smith, Wrightia 2: 98. 1960. **Fig. 1**; **Map 1**

Map. 1. *Spigelia anthelmia.*

Type: Hortus Uppsala, herb. Linnaeus 210.2 (LINN).

Heterotypic synonyms: *S. nervosa* Steud., Flora 26: 764. 1843; De Candolle, l.c. p. 560. Type: Suriname: Hostmann & Kappler 505 (isotypes: BM, 2 sheets, K, P, 3 sheets). Homotypic synonym: *S. anthelmia* L. var. *nervosa* (Steud.) Progel, l.c.

S. multispica Steud., l.c.; De Candolle, l.c. p. 560; Pulle, Enum. 373. 1906. Type: Suriname, Para District, Hostmann & Kappler 851a (isotypes: K, 2 sheets, P).

S. multispica Steud. var. *discolor* Progel, l.c. p. 263. Type: French Guiana: sin. loc., Poiteau July 1824 (K).

Annual herb, 2–60 (usually 20–50) cm high. Stems and each branch terminating with a whorl of 4 leaves and some inflorescences. Corolla lilac, pink, white, or tube white and lobes pale pink, with

Fig. 1. *Spigelia anthelmia:* a. Plant apex, ½ ×; b. Flower bud, 3 ×; c. Flower, 3 ×; d. Opened flower, 3 ×; e. Calyx with bracteole, 6 ×; f. Apex of sepal outside, 24 ×; g. Base of sepal inside, with colleters, 12 ×; h. Base of bract inside, with colleters, 12 ×; i. Base of leaf above, with colleters, 3 ×; j. Cupular base of capsule above, 6 ×; k. Idem laterally, 6 ×; m. and n. Anthers both sides, 12 ×; p. Pistil, 6 ×; q. Longitudinal section of ovary, 30 ×; r. Ovule, 60 ×; s. Fruit, 3 ×; t. Valve, 3 ×; u. Seed ventrally, 6 ×; v. Seed laterally, 6 ×; w. Portion of leaf margin above, 12 ×; y. Small plant, ½ ×; z. Plant apex, ½ ×. (a–w. Leeuwenberg 3210; y. Aruba, Stoffers 1836 (U); z. Suriname, Rombouts 624 (U)).

or without 5 pairs of longitudinal lines inside below the lobes, $2\frac{1}{2}$–$5\frac{1}{2}$ × as long as the calyx, 8–17 mm long, glabrous on both sides. Capsule green, composed of 2 ellipsoid parts, with an impressed line in the middle, conspicuously tuberculate, scabrous; the remaining base boat-shaped or nearly fusiform.

Stems erect, terete, unbranched or once or twice branched, glabrous, pale green, with one or two (sometimes more) pairs of leaves between the base and the apex; branches 1–2 together in the axils of the cauline and sometimes in the axils of the apical leaves. *Leaves* of apical whorl which consists of 2 decussate pairs close together, usually much larger than the cauline ones. Leaves of these pairs equal, but the pairs usually unequal; all sessile or sometimes petiolate; petiole up to 10 mm long; blade pale to medium green above, paler (slightly greyish-green) beneath, herbaceous, drying papyraceous, ovate-oblong, ovate-lanceolate, or oblong-lanceolate, 2–4 × as long as wide, 4–18 × 1.2–6 cm (cauline ones up to 10 × 3.5 cm), acuminate at the apex, more or less suddenly narrowed to the base or decurrent into the petiole, entire, sparingly puberulous to glabrous above, more puberulous to glabrous beneath. Stipules united, membranaceous, broadly triangular, about ½–1 × as long as wide, obtuse, entire, glabrous. *Inflorescences* terminal and in the axils of the whorled leaves, cincinnous, 1—several together, shorter or longer than the leaves. Peduncle very short, glabrous or sparsely puberulous like the axis. Bracts very small, sepal-like and of about the same size as the sepals. Flowers solitary, sessile or subsessile. *Sepals* pale green, free, unequal, linear-lanceolate, 7–12 × as long as wide, 2–5.5 × 0.3–0.5 mm, acuminate, entire, glabrous on both sides or outside sparsely puberulous, the largests up to 1½ × as long as the smallests. *Corolla* tube 6.5–15 mm long, 0.8–1 mm wide at the base, 2–3 mm at the throat, slightly contracted above the base, not ventricose; lobes equal, triangular, about 1.3 × as wide as long, 1.5–2.2 × 2–2.5 mm, entire. Stamens included; filaments glabrous, inserted slightly below the middle of the corolla tube; anthers glabrous, lanceolate, 4–6 × as long as wide, 1.2–1.7 × 0.25–0.3 mm, slightly sagittate at the base, obtuse at the apex. Ovary glabrous, globose or nearly so, 0.5–0.8 mm in diam., not or slightly narrowed into the style, with 2 somewhat impressed lines above the septum; style slightly exserted, pubescent above, glabrous beneath, shading off into the stigma, upper half deciduous, lower half persisting; stigma inconspicuous. *Capsule* 1.2–2 × as wide as long, 4–5 × 5–6 mm, obtuse at the base, 2-lobed at the apex; valves paler, glabrous, and glossy inside. *Seeds* obliquely ellipsoid or ovoid, dull, medium to dark brown, conspicuously tuberculate, about 1½ × as long as wide, 2–3 × 1.5–2 mm, glabrous. Embryo white, spathulate, 1.8–2 mm long, surrounded by white endosperm; cotyledons elliptic, about 1½ × as long as wide, 0.8–0.9 × 0.5–0.6 mm, rounded at the base and at the apex; rootlet 0.3 mm in diam., obtuse at the base.

Distribution: Indigenous in tropical America, from Mexico and Florida to Peru and Brazil. Naturalized in tropical Africa, especially in West Africa, and in Indonesia in Java and some neighbouring islands.

Ecology (in Africa): Waste places at low elevations.

GUINEA: Conakry, de Wit 6068 (WAG); ibid., Monod, rec. 15 Dec. 1946 (IFAN); ibid., Chillou 1453 (BR, COI, IFAN, K); Salesses, Chillou 1616 (BR, PRE).

SIERRA LEONE: Rokupr, Jordan 258 (K); Binkolo, Northern Province, Deighton 1284 (BM, K); Makump, Dawe 505 (K); ibid., Deighton 1408 (BM, K); Freetown, Deighton 2061 (K); Kortright, Freetown, Gledhill 26 (K); Kasawe Forest Reserve, King 268 (K); Njala, Pyne 5 (K); Bo District, Bo, Gardner 47 (BM), 76 (BM).

LIBERIA: Monrovia, Dinklage 2763 (B, P); ibid., Linder 1540 (GH, K); ibid., Voorhoeve 126 (WAG); Kakata, N.E. of Monrovia, Bequaert 189 (K).

IVORY COAST: Bouaké, Chevalier 34138 (P); Dimbokro, Chevalier 34103 (P); 64 km N. of Sassandra, 3 km N. of Niapidou, Leeuwenberg 3210 (GC, WAG); Sassandra, Dautiat (?), rec. 10 July 1948 (IFAN); Yapo, 50 km N. of Abidjan,

Roberty 15358 (G); near Divo, de Wit 6067 (WAG); Adiopodoumé, 17 km W. of Abidjan, Giovanetti (?) 177 (IFAN); ibid., Herb. I.D.E.R.T. 1553 (ABI); ibid., Leeuwenberg 1925 (BR, GC, K, LISC, MO, P, WAG, Z); ibid., Roberty 15750 (G).

GHANA: between Asokua and Kumasi, Darko 708 (BR, K); Kumasi, Roberty 12775 (G, Z); Dunkwa, Whiting 21 (K); Benso, Tarkwa District, Andoh F.H. 5516 (BR, K); Achimota, Ankrah Agric. 216 (K); ibid., Irvine 1446 (K); ibid., Morton G.C. 25317 (K); Labadi, Accra Plains, Anteson s.n. (GC); Legon Hill, Quarry Road, Adams 3500 (GC).

NIGERIA: Jebba, near Niger R., Hagerup 718 (K); Oyo Province, Ibadan District, Jones 753 (K); Ibadan, Coombe 122 (BR, K); ibid., Humbler 323 (K); ibid., Roberty 1631 (G); Lagos, Bels 57 (U); ibid., Chevalier 14065 (P); ibid., Dalziel 1042 (E, K); ibid., Dawoder (?) 31 (E); ibid., Dodd 432 (P); ibid., Stubbings 72 (K); Port Harcourt, Niger Delta, Maitland A (K).

CONGO: LEOPOLDVILLE: Kisantu, Callens 3654 (BR).

ETHIOPIA: S.W. of Jimma, Jimma Agr. Tech. School D 7 (K).

MADAGASCAR: sin. loc., Perrottet s.n. (G) (finding loc. doubtful).

The description is based upon the specimens cited above and the extra-African material of the following herbaria: K, L, P, U, WAG.

English name: Wormgrass.
French name: Brinvillière.

LINNAEUS gives the following citations with *Spigelia anthelmia:*

Arapabaca quadrifolia, fructu testiculato. Plum. gen. 11. (= Plumier, Nov. Pl. Amer. Gen. 11, t. 31. 1703); Barr. equin. 15. (= Barrère, Essai Hist. Nat. France Equinoxiale 1st. ed. 15. 1741; 2nd. ed. 15. 1749 (same text)).

Arapabaca brasiliensibus dicta planta. Marcgr. bras. 46. (= Piso & Marcgrav, Hist. Rer. Nat. Brasiliae 35. 1648, with figure of flowering and fruiting plant).

PLUMIER describes and figures the flower and the fruit of *Spigelia anthelmia.* PISO & MARCGRAV describe and figure an unbranched plant with a single inflorescence bearing flowers and fruits. BARRÈRE cites the phrase of PLUMIER with that of PISO & MARCGRAV as synonym. LINNAEUS gives after his citations a description in italics of a once branched specimen with two branches and solitary inflorescences at the apices of the stem and the branches. Therefore it is evident that he did not base his description on those of the cited authors, but that he saw living plants or herbarium material of this species.

In the Linnean Herbarium of the Linnean Society of London two specimens of *Spigelia anthelmia* are preserved. One much branched collected by Rolander in Suriname after the publication of the Species Plantarum in 1755. The other specimen is provided with the abbreviation H.U. which means Hortus Upsaliensis. This specimen agrees, though not completely, with his description. It bears two not entirely developed branches. The third specimen seen by LINNAEUS examined by the present author is preserved in the Stockholm Herbarium. This is unbranched. Of the three specimens discussed above the second one agrees the best with LINNAEUS's description and therefore here is considered the type.

Although it was nct easy to find out what specimen might be the type, there is no doubt about the identity of the species. All cited authors mean the same species to which belong undoubtedly all specimens discussed.

Remark: The other varieties cited by PROGEL which may be specifically different from *S. anthelmia* are not studied here.

Uses: Plant was used as anthelmintic, but dangerous by poisonous alkaloids. As it was introduced in Africa shortly ago nothing is known of use by Africans.

ACKNOWLEDGEMENTS

The author expresses his gratitude to the directors and curators of the herbaria cited in the two preceding papers on African *Loganiaceae* and of the Ghana Herbarium (GC), the Oxford Forest Herbarium (FHO), and the National Herbarium of Pretoria (PRE) for putting material at his disposal.

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TRANSLOCATION OF C^{14}-PHOTOSYNTHATES IN THE GRAFT MUSKMELON/CUCURBITA FICIFOLIA

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(*received September 22th,* 1961)

Introduction

The graft combination of muskmelon on *Cucurbita ficifolia* was shown by Wellensiek (1949) to be incompatible unless some foliage is maintained on the rootstock. Removing this stock foliage results in a rapid death of the whole combination; dying significantly starts in the stock. Because of its interesting nature, De Stigter (1956) studied this graft in more detail, including items like the relationships between number of stock leaves and scion growth, distribution patterns of starch, root formation in graft-cuttings, behaviour of double graft combinations and histological observations. On the basis of the results obtained, the conclusion was reached that the incompatibility in the absence of stock leaves is not due to a lack of union between the partners, but rather to the lack of a factor, a "specific substance", being produced by the leaves of the cucurbit stock only, but not by the melon scion, and necessary for the proper functioning of the stock's phloem.

From the above *résumé* it is evident that in one way or another, translocation of organic compounds must be involved. In order to check the above hypothesis, and to obtain a more detailed picture of the distribution of photosynthates in this graft, it was decided to study these processes by means of the radioactive carbon isotope C^{14}.

As our problem turned out to be more complex than it had at first appeared, it was felt necessary to reconsider the whole subject, opposing once again the two major points of view of our above-mentioned paper, *i.e.*, the purely structural, anatomical approach, and the more physiological one. In our opinion, the considerations to be developed apply to the physiology of grafted plants in general, and thus are not limited to our special case only.

Materials and methods

As experimental material, muskmelon, *Cucumis melo* L., var. Suikermeloen, and *Cucurbita ficifolia* Bouch. were used. A detailed account of raising the plants, and of the grafting techniques has been given in our previous paper.

As before, the various graft combinations will be indicated by symbols using the following abbreviations and signs:

M and F : muskmelon and *C. ficifolia*, respectively;
+ and —: with and without leaves on the stock, resp.;
*: place of application of C^{14}.

The young grafts were grown with two or three leaves on the stock. In experiments without stock leaves, these were removed one day before, or just prior to the administration of C^{14}.

Unless otherwise specified, the lowermost leaf of the melon scion was always treated, by exposing it to $C^{14}O_2$. The $C^{14}O_2$ was released from $BaC^{14}O_3$ by lactic acid, and circulated in a closed circuit containing up to six leaf chambers in a parallel arrangement. Thus, a varying number of plants, to a maximum of six, could be treated at the same time. As a rule, 15 μc of C^{14} were given per plant. The exposed leaves did not stay in the photosynthesis chambers for periods longer than one hour; the total duration of the translocating periods varied from a few hours to one day, or in some cases even two days.

At the end of the experimental period, the treated leaves were detached, and the plants cut into several pieces, to facilitate handling after drying. The treated leaves were loosely wrapped in tissue paper to prevent contamination of other parts. The plants were arranged in wire trays, quick-frozen with pulverized dry ice, and vacuum-dried at a temperature of about $-15°$ C; *cf.* YAMAGUCHI and CRAFTS (1958).

The dried plants were carefully flattened, mounted on 14″ × 17″ sheets of paper, and pressed. For autoradiographing, as for the freeze-drying, the procedures described by Yamaguchi and Crafts were followed. The film material used was Kodak No-Screen X-ray film, and the time of exposure 14 days.

For histological details a few microautoradiographs were made, employing a modified stripping-film technique. The material (short pieces of rootstock stem) was dehydrated by freeze-substitution in methyl-cellosolve, embedded in paraffin and sectioned at 15 microns thickness.

For the histological examination of phloem, the tannic acid — ferric chloride — lacmoid staining combination of CHEADLE, GIFFORD and ESAU (1953) was employed, using rootstock material preserved in 70 % ethyl alcohol.

In order to be able to observe and record root growth, and to make autoradiographs of undisturbed root systems, a special method of water culture was devised and successfully used. The unit for each individual plant of this set-up consisted of a shallow tray, sized as to accommodate a 14″ × 17″ piece of black nylon fabric on the bottom, and covered with a removable lid of a lath frame lined with thin polyethylene film. To exclude the light, and excessive heat, this lid was again covered with a piece of black cotton and a piece of cheese cloth, respectively. The nylon fabric on the bottom of the tray was soaked by Hoagland's culture solution continuously dripping down from two pieces of glass tubing introduced through small holes in the plant holder at one end of the tray. Between these two capillaries a plant was fitted in the plant holder, and its roots spread out on the nylon. Twelve trays were arranged in line, with a common supply; they were placed in a slightly slanting position, so that the excess of culture solution could flow off, to be collected and recirculated by means of a pump with float switch.

Apart from the periodic replenishing and renewal of the culture solution, the only maintenance of this system consisted of checking the glass capillaries from time to time. Root growth could be followed and recorded by making white-pencil marks on the black nylon. At the end of an experiment the plants were cut close to the root collar, and the root systems vacuum-dried while still attached to the nylon.

Results and preliminary discussion

The first item to be studied was the translocation and distribution of C^{14}-photosynthates in the absence and in the presence of stock leaves, or symbolically in M*/F— and in M*/F+, respectively. As can be seen from plates 1 and 2, the distribution patterns were remarkably alike: in neither case could any appreciable amount of radioactivity be observed in the root system, 24 hours after exposing one melon leaf to $C^{14}O_2$. Countings performed on thin transverse sections of rootstock stem were hardly, if at all, above background: only about 6 and $2\frac{1}{2}$ counts per minute for M*/F+ and M*/F—, resp., on a basis of two hours counting with a background of 27.2 c.p.m. Even after 48 hours the results were virtually the same. The bulk of downward translocation stopped right at the node above the graft union, only negligeable amounts travelling further downwards. Only in a very few cases, visible and measurable quantities of radioactivity did occur in the stock tissues and roots, both in M*/F— and in M*/F+.

The only difference between the two treatments, though not to be seen in the pictures, was that in M*/F— root growth had stopped completely, while in M*/F+ root growth was continuing normally. Apparently, in M*/F— a condition of incompatibility develops right after the stock leaves have been cut. As was shown before, De Stigter (1956), the sieve tubes of the stock show visible symptoms of collapse as early as one day after defoliation.

That in M*/F+ no, or no appreciable radioactivity is found in the roots, might be taken to mean that the roots are provided with all they need by their own leaves, and thus do not act as a "sink" with regard to the scion as a potential "source" of photosynthates (for the terms sink and source, see Crafts and Yamaguchi (1958)). Control experiments in which both scion and stock were *C. ficifolia*, revealed that in F*/F+ the roots were practically free from radioactivity, while in F*/F— great amounts of radioactivity were found in the roots very soon after the $C^{14}O_2$ was given. These results seem to confirm the view that in these grafts the roots are served only by their own leaves, if present. However, quite different results were obtained in other control experiments with grafts of muskmelon only; here the presence of stock leaves did not prevent the labelled photosynthates from entering the rootstock. Yet, here also, the presence of stock leaves had a suppressing effect, the stock tissues of M*/M+ containing twice as little radioactivity than did those of M*/M—; transverse sections produced 127 and 249 net counts per minute, and per section,

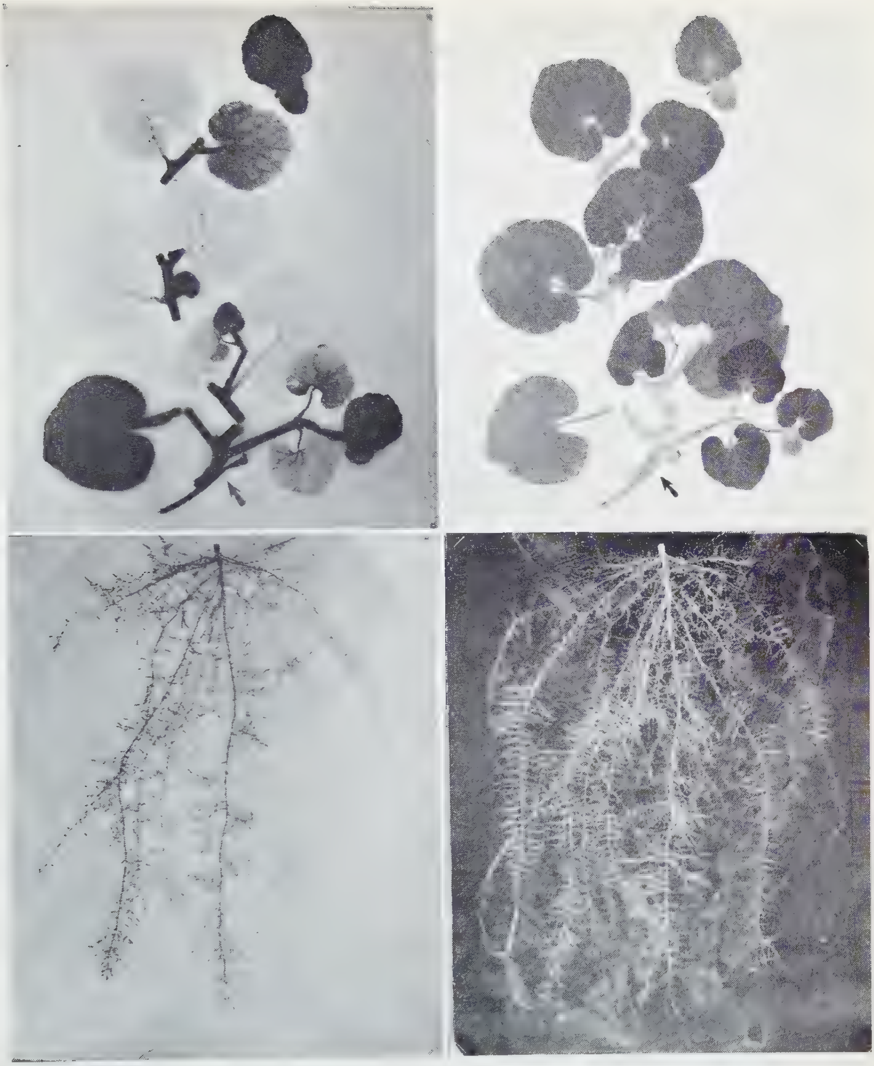


Plate 5. Melon/*Cucurbita ficifolia*, without stock leaves: M*/F—. Exposure to $C^{14}O_2$ (melon leaf at lower left) 10 days after stock shoot was removed (stump of shoot extends to upper right of arrow which indicates graft union). During these 10 days after removal of stock leaves and prior to exposure to $C^{14}O_2$, the plant went through a serious condition of incompatibility, followed by the onset of a gradual recovery. In the stage shown, recovery is most prominent in the new root growth, the above-ground parts still looking rather bad (this does not show up in the picture! . Especially the exposed melon leaf was still badly yellowish-green. In this recovering plant, the labelled photosynthates readily travelled into the roots, even in such quantities that the film had to be exposed much shorter than usually: only 1 day instead of 14 days; the latter period produced a fully over-exposed, blurred image. Note that complete, apparently dead, roots are missing in the autoradiograph.

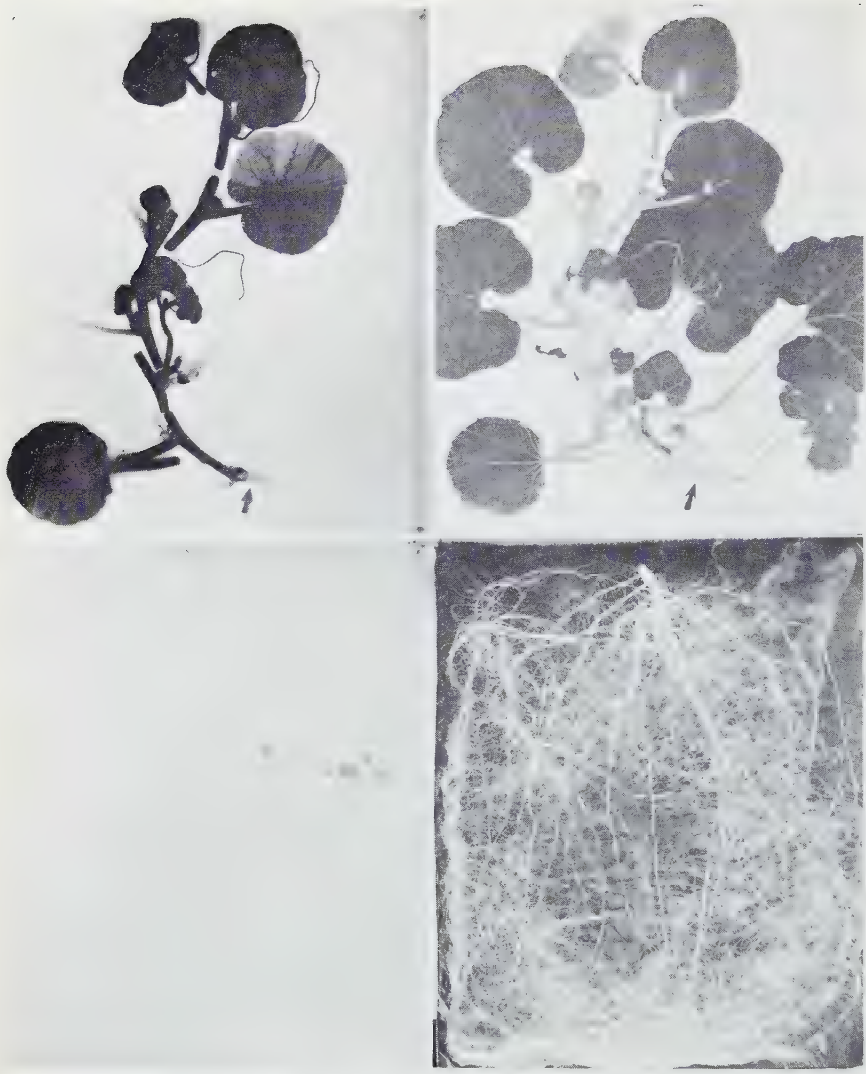


Plate 2. Melon/*Cucurbita ficifolia*, with stock leaves: M*/F+. April 2–3, 1959. Same details as plate 1, except for the stock shoot which occupies the lower right corner. Note that here, too, the roots are free from radioactivity (see text).

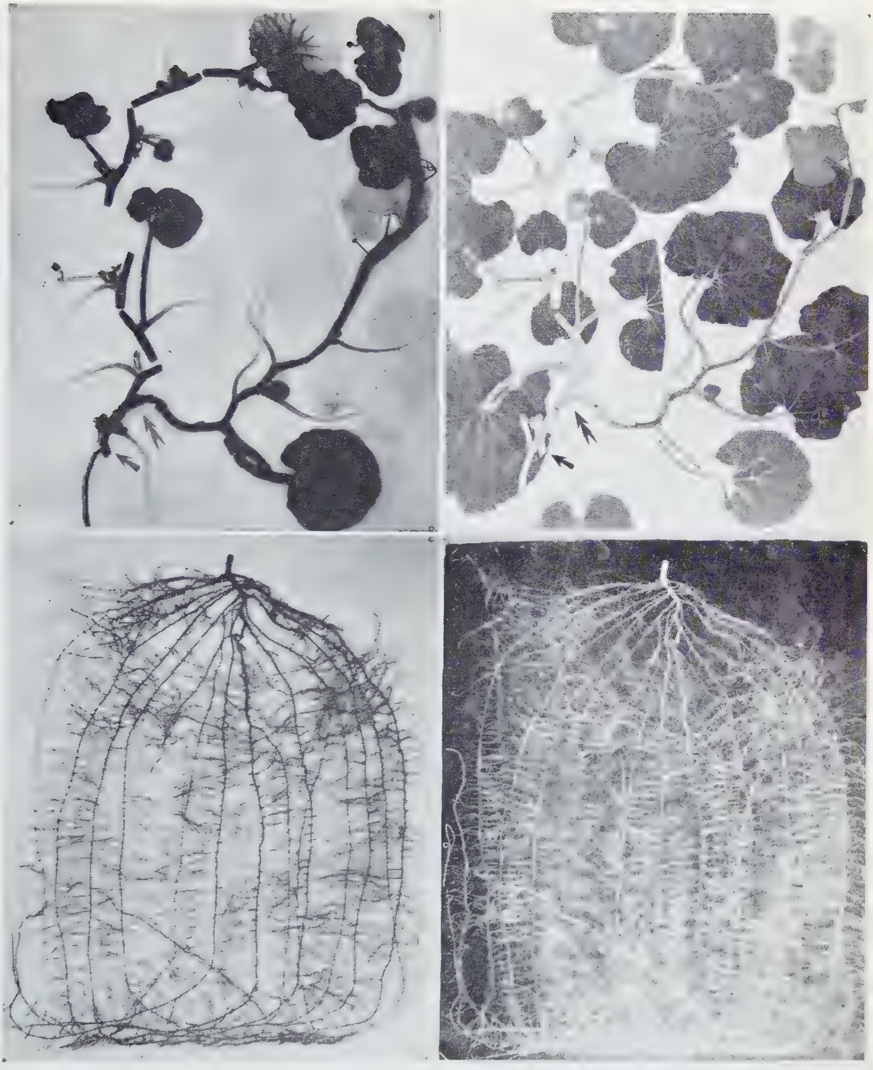


Plate 3. Melon/*Cucurbita ficifolia*, without stock leaves, but with another, laterally inserted *Cucurbita* shoot: F*/M/F—. April 6, 1959. Arrow: original graft union; stump of detached stock shoot extends to upper left. Winged arrow: second graft union, inserted *Cucurbita* shoot fills lower right part. Lowermost leaf of inserted shoot was exposed to $C^{14}O_2$, $\pm$ 30 μc. Duration of translocation and distribution period: 6 hours. In contrast with plates 1 and 2, radioactive compounds have travelled to the roots, now even passing two graft unions.

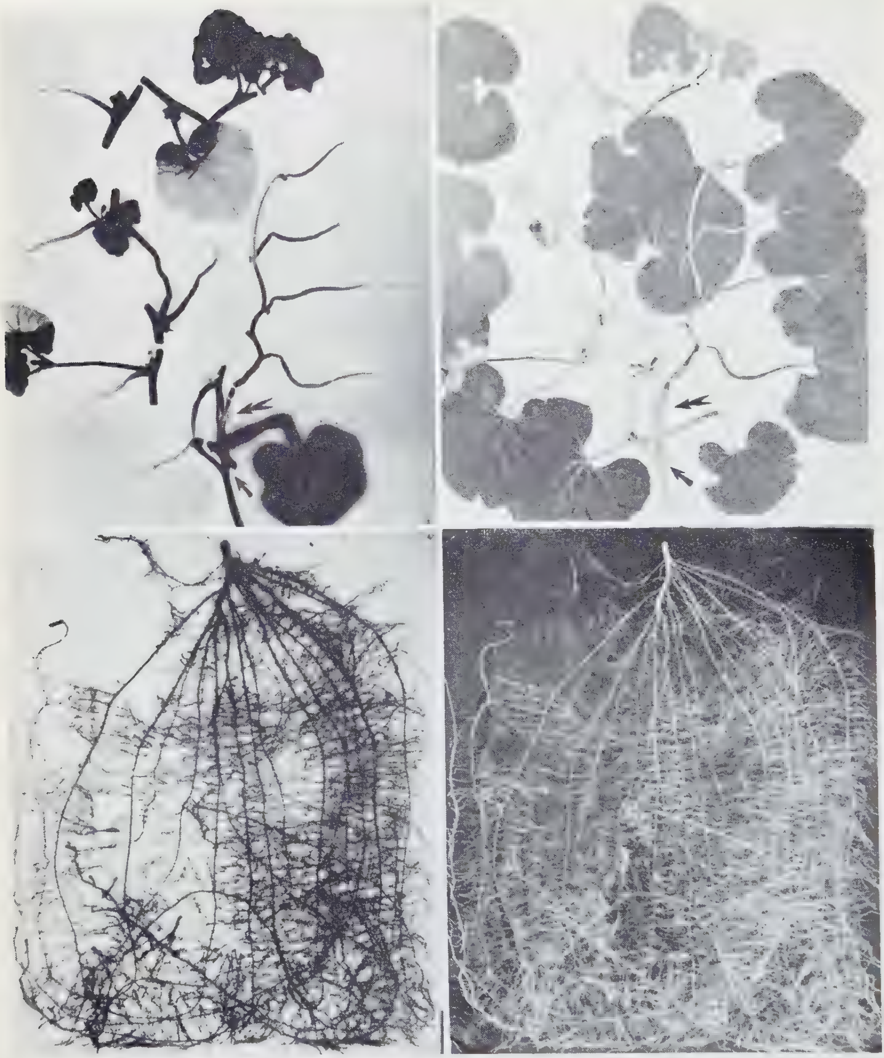


Plate 4. Same graft combination as in plate 3, but point of application of $C^{14}O_2$ is the leaf on the melon intermediate: F/M*/F . April 6, 1959. Results are the same as in plate 3, except that the roots contain still more radioactivity.

Translocation of C^{14}-photosynthates in the graft muskmelon/Cucurbita ficifolia.

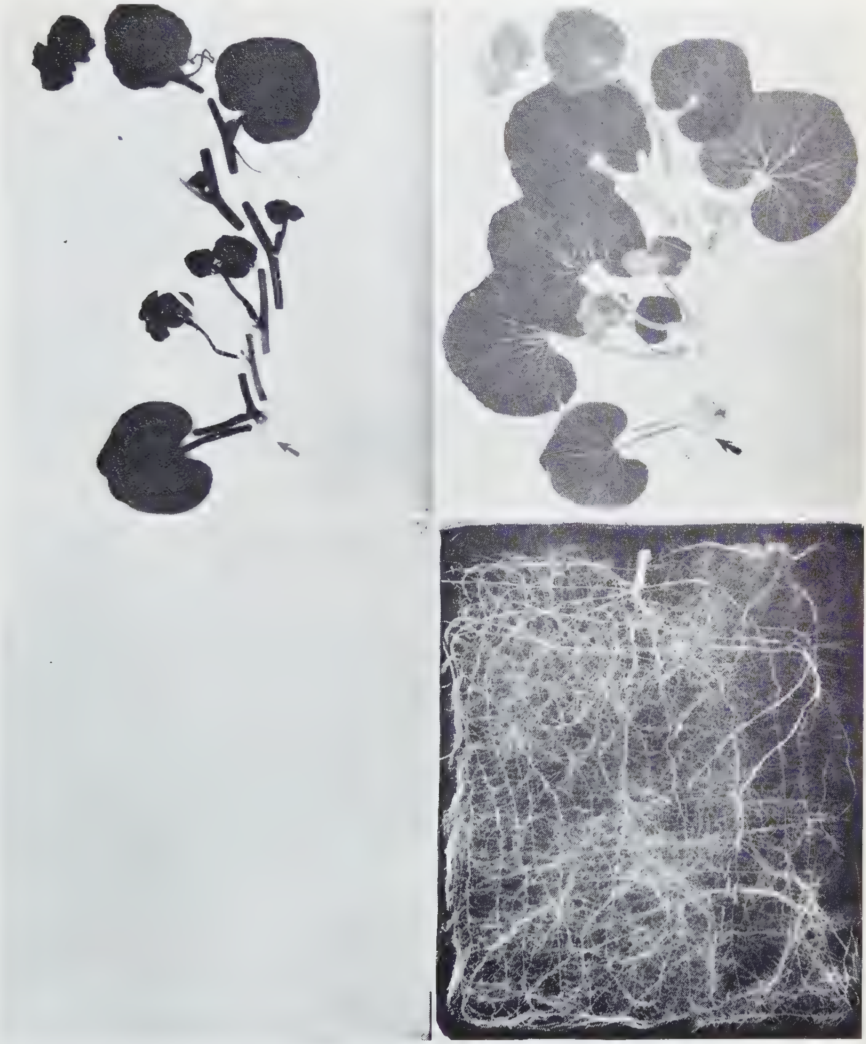
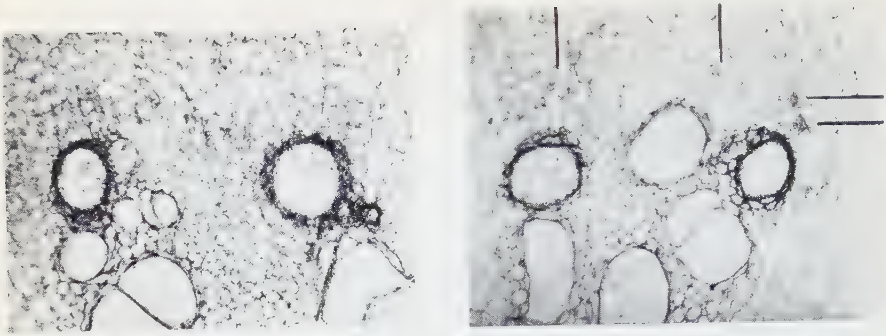
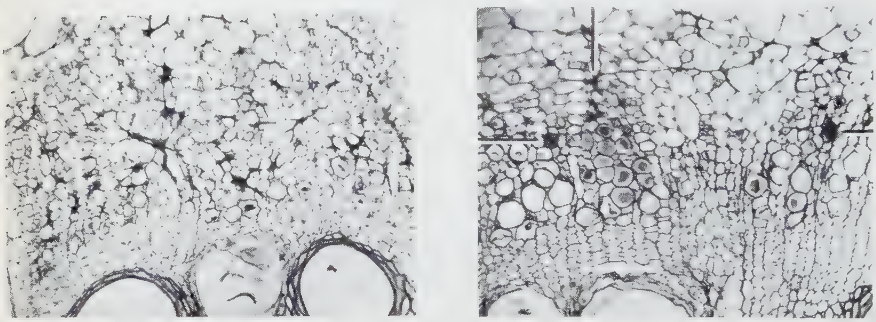


Plate 1. Melon/*Cucurbita ficifolia*, without stock leaves: M*/F , April 2 3, 1959. *Right:* plant and root system; *left:* autoradiographs of same. Graft union indicated by arrow. Stump of detached stock shoot extends to the upper right of arrow. Melon leaf at lower left was exposed to $C^{14}O_2$, ± 15 μc. Duration of translocation and distribution period: 24 hours. Note that downward translocation practically stops at the node above the graft union. Within the scion, C^{14}-compounds accumulate in parts which are still growing, and hence importing ("sinks"): top parts, axillary shoots, tendrils. As a contrast, full-grown leaves (exporting organs) hardly show up in the autoradiograph.



a *b*

Plate 6, *a* and *b*. Microautoradiographs of transverse sections from the stock of recovered M*/F— grafts. In both, radioactivity is concentrated in two of the youngest fully developed xylem vessels. In *b*, the horizontal lines indicate two newly-formed sieve tubes with high concentrations of radioactivity; the vertical lines point toward groups of collapsed sieve tubes.



c *d*

Plate 6, *c* and *d*. Micrographs of transverse sections from the stock of M/F— grafts. Plate 6, *c*: plant not yet recovered, but recovery just beginning. Most of the sieve tubes have completely collapsed, only near the cambium a few new sieve tubes are being formed. Plate 6, *d*: plant in a farther advanced stage of recovery. Vertical line points to a series of collapsed sieve tubes. Horizontal lines: sieve plates with solid (blue) callose deposits. Toward the cambium the sieve tubes become progressively wider, and the callose deposits progressively less heavy. The dark masses not filling the entire sieve tube are plugs of contracted sieve-tube contents stained dark brown by tannic acid and ferric chloride.

respectively. Although the results with both F*/F ± and M*/M ± can be described in the terms of source and sink, the marked difference in degree to which this explanation applies cannot be accountde for as yet.

It should be kept in mind that a sharp distinction must be made between cases in which the source-and-sink terminology applies, and cases in which a condition of incompatibility occurs, resulting in an absence of translocation. In the latter, the roots should fundamentally act as sinks, but in one way or another, *i.e.*, structurally or functionally, the route between source and sink is blocked.

Returning to the failure of M*/F— to show any appreciable translocation across the graft union, the question should be raised whether there is a lack of union between the partners, at least as far as the phloems are concerned, the direct xylem connections having been definitely proven, De Stigter (1956). In this connection it is relevant to refer to McClintock (1948) who found a discontinuity of the phloems in the graft combination of peach on Marianna plum rootstock. This graft, too, will stay alive if some branches with leaves are maintained on the stock.

In order to check the M/F phloem union, double grafts were made by inserting a cucurbit seedling laterally into the stem of the melon scion. After this second graft had established itself, the stock leaves were removed, and $C^{14}O_2$ was administered to the lowermost leaf of the cucurbit insertion. Plate 3 shows that in this F*/M/F— combination photosynthates did travel downwards into the roots, now even passing two graft unions. The conclusion can only be that the M/F graft union is very well able to function properly. The possibility should be considered, however, that only cucurbit photosynthates are "admitted" by the cucurbit rootstock tissues. To find this out, the same double graft combination was used, but now the leaf on the melon intermediate was exposed to $C^{14}O_2$. From plate 4 it is evident that here, too, considerable amounts of radioactivity have travelled into the roots. Thus the possibility of specific photosynthates has been ruled out.

Up to this point everything appears to support our original hypothesis that some factor originating from the cucurbit leaves is required to keep the stock phloem functioning, so that, in its absence the sieve tubes immediately stop functioning and collapse. A more prolonged observation of M/F— plants, however, showed that matters are still more complex. In our special system of water culture the plants started showing every symptom of incompatibility after removal of the stock leaves: complete cessation of root growth, die-back of large parts of roots, stagnation of top growth, yellowing of leaves, sieve tube collapse in the stock (plate 6*c*), but the plants just failed to die altogether, as they invariably will do when grown in soil. Instead, after some 8–10 days, tiny lateral rootlets began to appear higher up the main roots, so tiny indeed that at first they were taken for fungal outgrowths. Gradually these rootlets began to grow, after some time at

ever increasing speed. Shoot growth also resumed, in short, the plants showed a gradual but complete recovery. Once this process of recovery has started, C^{14}-photosynthates readily travel downwards across the graft union, as can be seen from plate 5. The plant from which this autoradiograph was made, even contained so much radioactivity in its roots that a film exposure of only one day was sufficient to produce the image presented here; the normal period of 14 days which was first tried, gave a completely over-exposed, blurred image.

Microautoradiographs, some details of which are reproduced in plate 6, *a* and *b*, showed that the bulk of the radioactivity in the stock is present in one or more of the youngest fully developed xylem vessels, some activity also being found in the newly-formed sieve tubes, and in the starch, if present.

Histological examination of recovered plants revealed a wide range of phloem conditions in the stock, from fully collapsed sieve tubes in the older parts, *via* stages of relatively narrow sieve tubes with variable, frequently heavy callose deposits, to big healthy sieve tubes with wide sieve perforations and little callose, near the cambium; see plate 6*d*.

General discussion

From the experimental results it is evident that one of the main questions of our problem concerns the exact nature and functioning of the graft union. As a first approach, histological examination showed, De Stigter (1956), that in the course of the process of uniting, a cambium is formed, continuous with the cambia of stock and scion. This cambium accomplishes the definitive union between stock and scion. Direct continuity of the xylems can be easily observed, even macroscopically, but is much more difficult to demonstrate for the phloems. As a result, the phloem continuity has not strictly been proven, but our conclusion was that "there is no reason to doubt the existence of a direct phloem connection between the melon top and the cucurbit stock". This phloem continuity was further substantiated by the remarkably good growth of the double graft F/M/F—. In our present study this has again been confirmed by F*/M/F— and F/M*/F—, in both of which rapid downward translocation took place irrespective of whether the labelled photosynthates originated from the cucurbit top or from the melon intermediate.

The results of these double graft experiments, with apparently well functioning unions, were considered to be conclusive proof that also the simple M/F graft union is perfectly in order, anatomically. Still, however, against this conclusion the criticism might be raised that the M/F graft union is *not* fully perfect originally, but that the laterally inserted cucurbit shoot in F/M/F— induces this first union to improve to such an extent as to become fully capable of functioning. While such an action of the second graft should not be considered impossible, it must be borne in mind that even the single M/F— graft turned out to be able to achieve this improvement (supposed we justly use this term), without the help of a second graft, as shown by its full

recovery. This spontaneous recovery of M/F—, now, is a most unexpected thing. If we continue, for the moment, thinking in terms of an imperfect graft union, this recovery implies that only defoliation of the stock will put the plant to complete its graft union (after having been on the verge of death). In the presence of stock leaves it would never have achieved this completion, for defoliation of the stock will bring about the full range of incompatibility reactions at any time, no matter how young or old the graft is. The definitive answer to the questions involved awaits a much more detailed and scrupulous examination of the graft union, at the exact site of transition. For the time being, however, we do not consider the "incomplete phloem hypothesis" to be very probable.

The alternative, by far to be preferred, is to approach the problem from a physiological point of view, by assuming that the biochemical functions of stock and scion are not fully complementary, or that a condition of biochemical unbalance exists. Our original hypothesis of a "specific substance", required for the proper functioning of the rootstock's translocatory system would fit this view. To reconcile this hypothesis with the spontaneous recovery of M/F—, we have to assume that the stock has to pass through a process of adaptation, as a result of which it becomes able to synthesize the "translocation factor" from the melon's supply of photosynthates. If this is true, it seems reasonable to assume that the stock will need some time to build up an enzymatic apparatus of sufficient capacity. This would account for the recovery being only slow and gradual.

The above considerations will suffice to show that in grafted plants one should not limit himself to thinking about the graft union as a mere mechanical barrier. Moreover, in translocation studies quite other factors may be involved, as demonstrated by the different behaviour of our F/F and M/M control grafts. The relative amounts of foliage present on scion and stock will greatly influence the distribution pattern of compounds applied to either one of the partners. These effects are best described as resulting from the degree to which the various parts act as "sources" and "sinks" with regard to each other. One should be aware of this in interpreting the results of DILOV, POPOV and KASSABONEVA (1959) on the distribution of P^{32} in grafted melons and pumpkins. They themselves ascribed their findings to a "barrier effect" exersized by the graft union. It is interesting to note that they, with P^{32}, do not mention any complete or nearly complete absence of downward translocation in their melon/pumpkin grafts like we did in ours with C^{14}. Still, of all the combinations made, the roots of melon*/pumpkin contained the smallest relative amount of radioactivity.

Finally, we should discuss why M/F— has always appeared to be completely incompatible, while it is now evident that the very same combination can make quite satisfactory growth. Our explanation is simply this, that in soil the weakened roots cannot withstand the

action of microorganisms attacking them *en masse*, while in our system of water culture conditions are much more favourable for survival and recovery.

SUMMARY

In this study, using autoradiographic techniques, our attention was primarily directed to the question whether or not, and under which conditions, the scion's labelled photosynthates would pass into the rootstock. Until recently, in the absence of stock leaves the melon/*Cucurbita* graft appeared to be completely incompatible. In a specially devised system of water culture, this turned out not to be true, however, since stock-defoliated grafts after an extremely critical period are able to recover gradually but completely. From then on they show a remarkably good growth. Microorganisms attacking the roots are taken to be responsible for recovery not taking place if the grafts are grown in soil.

Trying to account for this apparent incompatibility and subsequent recovery, the problem should be approached from different angles. The one way of approach concentrates upon the degree of perfection of the graft union, in the anatomical sense. The other major line of thought is biochemical in nature, and considers the question how far the biochemical functions of stock and scion are complementary, or balanced.

For the present, it seems justified to consider the graft union to be anatomically perfect. The "temporary incompatibility", then, should be due to the absence of a "translocation factor". The subsequent recovery can be visualized to be due to a process of biochemical adaptation.

Looking upon a graft union as a mere mechanical barrier in translocation studies, is to be critisized, the more so since still other factors may be involved, such as the degree to which the various parts of the graft act as "sources" and "sinks" with regard to each other.

ACKNOWLEDGEMENTS

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BRIEF REPORTS

Czechoslovak Botanical Society (Societas Botanica Čechoslovaca)

The Czechoslovak Botanical Society (Societas Botanica Čechoslovaca) takes the pleasure to inform you that a "Jubilee Congress of Czechoslovak Botanists" will be arranged to celebrate the 50th anniversary of its foundation. The Congress will take place in Prague beginning July 1st till July 8th, 1962, and it is hoped to invite guests from abroad. The program-item will be *Experimental Taxonomy*.

For this special occasion a double number of Preslia will be published containing the main reports. During the Congress excursions will be arranged to botanically and culturally interesting regions of Southern, Western and Central Bohemia.

The Congress-fee will be \$ 7 and should be sent in together with the definite application by March 1st, 1962. (For your information quote the prices of the daily pension which are in hotels de luxe \$ 8–\$ 10, in hotels "A" \$ 5.50–\$ 7 and in hotels "B" \$ 4–\$ 5). The prices of all excursions including board and lodging will be about \$ 30.

The Czechoslovak Botanical Society takes the liberty to invite you to participate in this "Jubilee Congress of Czechoslovak Botanists" and would be grateful if you could inform and invite your colleagues or members of your institution working in the field of Experimental Taxonomy. (The preliminary application should be sent to us by September 1st, 1961). The detailed program and further information will be sent when definite applications will be in. The charge for board and lodging as well as the excursions should be sent in together with the Congress-fee.

Address: Societas Botanica Čechoslovaca, Benátská 2, Praha 2.

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New taxa and new combinations in **bold face.** The names from the lists of plant species in "J. H. Schuurmans Stekhoven, Das Frühlingsbild des Diplotaxidetum ercucoidis", p. 327–334, are not included.